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PHYTOPATHOLOGY

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JULY, 1934

IDENTIFICATION OF CELERY VIRUS 1, THE CAUSE OF SOUTHERN CELERY MOSAIC

F. L. WELLMAN[1]

(Accepted for publication September 16, 1933)

It is with pleasure that the writer thanks the University of Wisconsin, Madison, Wisconsin, the city of Sanford, Florida, and the Boyce Thompson Institute for Plant Research, Yonkers, New York, for furnishing laboratory and greenhouse facilities without which many points reported in this paper could not easily have been discovered.

INTRODUCTION

The fact that diseases occurred that were caused not only by fungi and bacteria but also by viruses was first demonstrated before the year 1890. Since that time a large number of related maladies with their host ranges have been reported on both animals and plants. With phytopathological literature so filled as it is with data relative to these virus troubles, it becomes increasingly evident that mere report of a plant virus, even accompanied with an account of symptoms and its importance on the host, is not sufficient to place it in the category of a new and separate disease.

For over ten years a mosaic disease of celery has caused increasingly severe losses in Florida trucking areas, and has also been reported (5) from California, Wisconsin, Ohio, and New York, though it apparently has caused little damage in these latter States. The disease was reported from Florida in 1924 by Foster and Weber (7), but no experimental work had been conducted at that time. In 1929 a study was made of the disease by Doolittle (3) and it was continued in 1930 by Doolittle and Wellman (5). The last two authors have shown that it is a true mosaic transmitted both by juice inoculation and by the melon aphis, *Aphis gossypii* Glov., and was found to occur on a monocotyledonous weed, *Commelina nudiflora* L. Wellman continued work on this problem in the fall of 1930 and by 1932 had found (23) that eradication of this monocotyledonous weed host in the

[1] The writer thanks Dr. F. O. Holmes who gave him time, enthusiastic interest, and the benefit of broad experience on virus studies while at the Boyce Thompson Institute. Likewise, acknowledgment is due Dr. S. P. Doolittle for his interest in the problem and also Dr. J. Johnson for much constructive criticism.

vicinity of the fields was effective in eliminating in large measure losses due to the mosaic in celery fields.

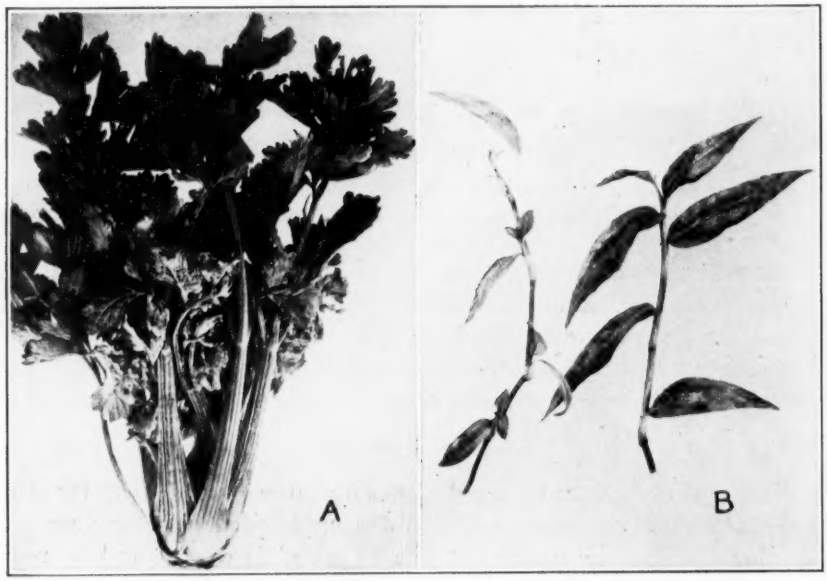

FIG. 1. Southern celery-mosaic symptoms on Golden Self Blanching celery and on the weed host, *Commelina nudiflora*. A. Mosaic-diseased celery plant at harvest time, cut off at the ground line, and stripped as for market. Such a plant apparently is only half as tall as a healthy individual, the petioles, instead of being straight, turgid, and white, are shrivelled and discolored, and there is a curvature of both petioles and leaf rachises. The leaflets are brilliantly mottled, somewhat savoyed, and slightly twisted, but they are not upright in growth habit nor do filiformities occur as is the case in plants infected with common cucumber mosaic virus. (Compare with Fig. 5, B.) B. On Commelina the southern celery-mosaic virus causes an irregularly watered type of mottling pattern of an indistinctly marbled or moiré-like appearance. It is only rarely that such leaf malformations occur on Commelina as are shown on the left branch in this figure.

The southern celery mosaic is characterized by leaf mottling and sunken discolored streaks on leaf stalks, but no filiformities or "shoe string" effects are produced on leaflets (Fig. 1, A). As will be pointed out later in this paper, these leaflet malformations are common symptoms produced either by Doolittle's (2) cucumber-mosaic virus or the tobacco ring-spot virus described by Wingard (24). These leaf malformations are always spoken of in descriptions of symptoms on mosaic-infected celery plants by Poole (19), Elmer (6), and Harvey (9). Johnson and Grant (16), using *Cucumber virus 1*, Doolittle, have proved that the common cucumber mosaic virus is readily transmitted to celery. From the relative abundance of the common

cucumber virus and the symptom expressions on diseased celery described in their publications, it is believed that Poole, Elmer, and Harvey probably were working with *Cucumber virus 1.*

Preliminary cross-inoculation studies by Doolittle and Wellman (5) with the southern celery-mosaic virus showed that it was transmissible to celery, cucumber, tomato, tobacco, and *Physalis pubescens,* and that it was successfully obtained from naturally infected celery, *Commelina nudiflora,* and *Physalis lagascae.* The symptoms on some of those hosts were somewhat similar to those caused by common cucumber-mosaic virus, and in some respects of other known virus diseases, notably, tobacco ring-spot. In view of these facts and the marked economic importance of the disease, it was evident that detailed studies of properties and identity of the causal virus were essential to the development of adequate control measures. In 1932 Wellman (23) had carried studies far enough to state the belief that the southern celery mosaic was probably caused by a virus distinct from the one causing common cucumber mosaic. As a result of these and later studies herewith reported, it is clear that the virus causing southern celery mosaic is specifically different from any heretofore described.

MATERIALS AND METHODS

Eight strains of viruses were used in these experiments for identification of the southern celery-mosaic virus and were from known sources. These have been listed in tabular form and are given in table 1. All viruses were acquired from living diseased plants obtained from the workers who so kindly furnished them, and, after being transferred to healthy greenhouse plants, they were checked as to symptoms produced on hosts and characteristic properties before being used as comparative material.

The original sources of southern celery-mosaic virus used in this work were a mosaic-infected celery plant and plants of the weed (*Commelina nudiflora*) obtained in an area of 10 square feet along the edge of a naturally diseased field in the Sanford, Florida, trucking district. Investigations soon proved that the weeds and cultivated plants were infected with the same virus. It has since been retained by continuous reinoculation methods. It has been carried in growing tobacco, cucumber, celery, and Commelina plants, and has been found to remain unchanged in its characteristics in any of these hosts through three years of such cultivation.

All of the earlier juice inoculation work reported by Doolittle and Wellman (5) was done by means of needle inoculation methods as described by Johnson (15), Doolittle (2), and many others. Using this technique it was possible to obtain a fair degree of transmission. However, in many cases such as dilution and thermal death-point studies, this technique was not satisfactory because erratic results were obtained, which were ex-

TABLE 1.—*Viruses used in comparative studies and identification of the southern celery-mosaic virus*

Virus	Name of disease	Authority	Source of virus	Date secured
Ring-spot	Tobacco ring-spot	Wingard (24)[a]	Wingard and Henderson (on tobacco)[b]	August, 1931
Tobacco mottle	Latent or "healthy" potato	Johnson (15)	Schultz (on Green Mountain potato)[b]	July, 1932
Cucumber virus 1	Common cucumber mosaic	Doolittle (2)	Doolittle (on cucumber)	October, 1930
" " "	" " "	"	Johnson, J. (on tobacco)[b]	April, 1931
Tobacco virus 1	Ordinary tobacco mosaic	Allard (1)	Doolittle (on tobacco)[b]	January, 1931
" " "	" " "	Johnson, J. (15)	Holmes (on tobacco)	June, 1932
Celery virus 1[c]	Southern celery mosaic[c]	Loc. cit.	Edge of Florida celery field (on celery)[b]	November, 1930
" " "	" " "	" "	Edge of Florida celery field (on *Commelina nudiflora*)	October, 1930

[a] See "Literature Cited."
[b] The strain used in principal comparative studies.
[c] Name given at close of studies.

plainable only on the basis of grossness of method. Attention was therefore turned to finding a more delicate measure of infectivity of variously treated inocula. Comparative experiments with tobacco, cucumber, and celery plants showed that Holmes' wiping method of inoculation (12) was superior to methods of injection, needle prick inoculations, and leaf mutilations.

Larger percentages of infection were commonly obtained by this method and in many cases of extreme virus dilution, where no infection was obtainable by other means, a high percentage of plants succumbed to the virus when the inoculum was applied by the wiping method. A search was then instituted to discover a host that, when inoculated, would produce primary lesions, such as were obtained on beans by Price (20) and on *Nicotiana glutinosa* by Holmes (12), when treated with the ordinary tobacco virus. Inoculations were made on a large number of hosts susceptible to the southern celery-mosaic virus and it was found that well expanded cotyledons of cucumber (White Spine variety) developed small, typical virus lesions that could be counted. The results obtained were consistent and comparatively satisfactory.

The wiping method of inoculation was used on cotyledons of cucumber seedlings developed from 10 to 15 days after planting seeds. The cotyledons were green and fully expanded, and the true leaves were small and in the form of a loosely folded rapidly expanding bud. Care was taken to have the plants in a fully turgid condition at the time of inoculation. A small square of cheesecloth was wetted with the inoculum and held in the right hand, while the fingers of the left supported the cotyledon to be wiped. This operation consisted of 10 gentle but firm strokes on each cotyledon from the stem end to the tip in such a way as to cover the cotyledon surface at each stroke. Cotyledons are very tender, brittle, and easily broken so some care must be exercised to avoid undue injury to the rubbed surfaces. After inoculation, about 3 to 5 minutes were allowed to elapse and the cotyledons were then washed with a jet of water to remove excessive materials, which if allowed to remain, may produce a general necrosis of the cotyledons. This latter washing has been studied and seems to have no depressing effect on the numbers of lesions produced. After each series of plants was inoculated the hands were always washed with soap and water and dried.

Development of primary lesions on cucumber cotyledons (Fig. 2) ordinarily takes 3 to 7 days, depending upon conditions. Counts were made by observing the underside of cotyledons, since an occasional lesion could be found on this surface, that was practically invisible from the upper side. Although the inoculum is applied to the upper side of the cotyledons, the undersurfaces are often the most distinctly marked and have shallow, dark

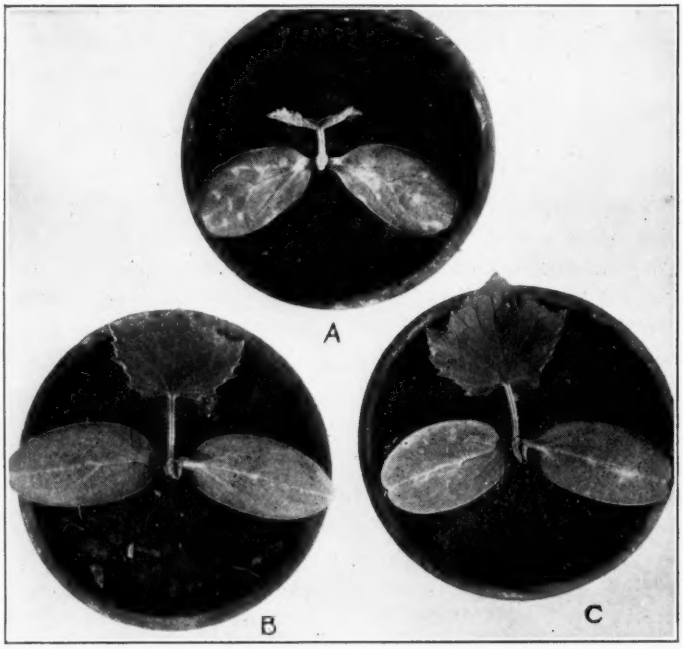

FIG. 2. Appearance of primary lesions of southern celery-mosaic virus on cucumber (White Spine) seedlings. A. Seedling infected by inoculating upper surface of cotyledons with juice from a celery plant diseased with the southern celery-mosaic virus. Note that 16 primary lesions are visible when these 2 cotyledons are observed from the upper surface. When these same cotyledons were turned over they were found to have a total of 19 primary lesions. Note also that the central bud seems to be tightly folded, the first true leaf has bent down and is dwarfed, and the infected cotyledons are drooping. B. Seedling whose cotyledons were inoculated with juice from a mosaic-free celery plant. Note that cotyledons appear turgid, are held in normal horizontal position, the first true leaf is expanding, and the bud containing the succeeding leaves is unfolding. C. Untouched, control seedling.

green, circular pits at the point of development of each primary lesion. Counts were made as promptly as possible after the lesions appeared, since they soon develop a necrotic condition that spreads, extends into the surrounding portions of the cotyledon, and renders the obtaining of numerical data uncertain.

It was found that the type of lesions and systemic symptoms developed by this virus (Fig. 1, A) is characteristic and may be relied upon for a criterion as to whether or not the celery virus is present. The virus multiplies in the cotyledons, and inoculations with juice from cucumber seedlings thus affected have regularly produced typical southern celery mosaic on celery.

Most of the work on properties has been done under all manner of seasonal conditions and in greenhouses located in widely separate States, Florida, Wisconsin, New York, and Virginia. Wherever the work has been done and under all conditions encountered the results have been characteristic.

EXPERIMENTAL RESULTS

PROPERTY STUDIES[2]

Filterability of the Celery Virus. Filtration experiments were not extensive. The fine grade "W" Berkefeld filters used were prepared, being washed and soaked in several changes of sterile distilled water. The filter candles were then set up in suction flasks, with a glass test tube so arranged in each flask that the filtrate would drip into the tube. These set-ups were then steam-sterilized, allowed to cool in a dust-free atmosphere, and finally attached with an intervening trap flask to a water pump which used regular city-water pressure to produce vacuum.

Eleven filtration tests were made using infective juice from plants of several ages. From 75 to 100 cc. of juice was obtained for each experiment and after being expressed from the diseased plants was strained through a double thickness of cheesecloth and poured over the filter candle. Filtration was fairly slow, but, within an hour after starting the suction pump, sufficient filtrate was obtained under aseptic conditions for tests to be made as to presence of both bacteria and virus. Regular bacteriological technique was used to test filtrates and unfiltered extracts for the presence of bacteria. Loopfuls of the liquids were plated out on neutral potato-dextrose and nutrient agars, and were also used to inoculate tubes of nutrient broth, which were incubated at between 24° and 28° C. for 14 days. In all, over 200 tubes and plates of media were inoculated in these tests. After these transfers, the remaining liquid was used to study the presence of virus. Small squares of sterilized cheesecloth were moistened with the remaining portions of filtrate and unfiltered extract and tobacco seedlings inoculated by the wiping method. From 5 to 15 plants were inoculated with the filtered and unfiltered juices and observed for 3 weeks before being discarded.

In the accompanying table 2 results from 7 representative experiments are listed. It will be seen that in these experiments all samples of plant juices before filtration contained bacteria and none after filtration. In 2 experiments (Table 2, Expts. 3 and 7) juice from diseased cucumber and tobacco plants, inoculated while quite large, was used. The cucumber

[2] The writer acknowledges the help of Dr. T. J. Grant, acting as collaborator in the U. S. Department of Agriculture, at Madison, Wisconsin, who independently corroborated many of his findings with regard to the properties of the southern celery-mosaic virus. However, the author takes full responsibility for all data reported and any errors are of his own derivation.

TABLE 2.—*Results of studies on filtration of southern celery-mosaic virus through fine ("W") Berkefeld filters, using Broadleaf tobacco seedlings as test plants for presence of virus, and potato and nutrient agars and nutrient broth to test for presence of bacteria*

Expt. no.	Inoculum		Presence of bacteria	No. mosaic plants
	Source	Treatment		
1	Old tobacco, mild symptom	Unfiltered	Present	15[a]
	" "	Filtered	Absent	0
2	" "	Unfiltered	Present	14
	" "	Filtered	Absent	0
3	Old cucumber,[b] severe symptom	Unfiltered	Present	15
	" "	Filtered	Absent	3
4	Young cucumber, "	Unfiltered	Present	15
	" "	Filtered	Absent	15
5	Seedling cucumber, "	Unfiltered	Present	15
	" "	Filtered	Absent	15
6	" "	Unfiltered	Present	15
	" "	Filtered	Absent	15
7	Old tobacco,[c] "	Unfiltered	Present	13
	" "	Filtered	Absent	2

[a] Numbers in this column represent total plants diseased from 15 inoculated by the wiping method.

[b] These plants were inoculated when past seedling stage and after the fifth true leaf was expanded.

[c] These plants were inoculated after the seedling stage and when 10 leaves were well formed.

juice (Table 2, Expt. 3), before filtration, produced 100 per cent diseased plants, or 15 diseased of 15 inoculated. After filtration, infection was reduced to 20 per cent, or 3 diseased of 15 inoculated. The tobacco juice (Table 2, Expt. 7), before filtration, produced 87 per cent infection, or 13 diseased of 15 inoculated, and after filtering infection was reduced to 13 per cent, or 2 diseased of 15 inoculated. Three experiments (Table 2, Expts. 4, 5, and 6) were performed using juice from cucumber seedlings 6 days after inoculation, which was the day serious systemic infection first appeared. Non-filtered plant juices were highly infective, giving 100 per cent of severe disease in all cases. After filtration these juices were still infective producing 100 per cent disease in the 3 experiments, but systemic symptoms on test tobacco seedlings were delayed in appearance from 1 to 3 days, which indicated a lessened concentration of virus in the filtered inocula. In 2 experiments, listed as numbers 1 and 2 in table 2, the virus was obtained from tobacco plants that had been systemically diseased for over 30 days. Mosaic symptoms in these plants were mild. These juices were good inocula before filtration, but after filtering they were apparently

free from the virus. These studies were carried further in subsequent experiments not represented in the accompanying table. In 3 cases mosaic-infected celery plants were used as sources of inoculum in which filterability of the virus in celery juice also was proved. Of 17 tobacco plants inoculated with bacteria-free filtrate from diseased celery plants, 5 developed symptoms typical of the southern celery-mosaic virus. The unfiltered juice produced 100 per cent disease in 17 control plants inoculated.

It would appear in filtration studies of this virus that infective filtrates are more readily obtained from juices containing a high concentration of the virus (Table 2, Expts. 4, 5, and 6) than from those juices where the virus concentration is low. In later studies expressed juice from severely diseased cucumber seedlings, such as those used in the experiments just cited, produced a total of over 150 primary lesions on 10 cucumber cotyledons. Juice from old tobacco plants, such as that used in experiments 3 and 7, listed in table 2, produced about 30 to 60 lesions.

Thermal Death Point. A common factor considered in determining differences in virus characteristics is that of the temperature at which the infective juice loses its disease-producing qualities. Because of its convenience as a term and its analogy in bacteriological studies, this induced inactivation of the virus by heating is called "death" in this discussion. The technique used by Grant and described in a paper by Johnson and Grant (16) was followed in these studies. The experiments were repeated over a period of several months. The work covered spring, summer, and fall growing conditions; and celery, cucumber, and tobacco plants were used both as test hosts and as sources of inoculum. With minor and expected deviations the results from all tests were markedly alike. (Table 3.)

It was found that occasional samples of viruliferous juices remained infective after treatment at 75° C. for 10 minutes, but in all cases the virus was dead in samples treated at 80°. It is probable that the ultimate point of death is no more important than the point at which first evidences of reduced infection result. The lower ranges of reduced infection with the virus apparently start a little below 50°. The lowest temperature at which a large reduction of virus infection results is below 65°. Freezing the virus seems to have little or no deleterious effect on its infective properties.

Tolerance to Dilution. In studying the dilutions at which juices of plants affected with southern celery mosaic would still remain infective, it was found that considerable variation existed dependent, apparently, upon type of host, succulence of plant, and vigor of disease development. Young diseased plants of cucumber, tobacco, celery, and *Commelina nudiflora* were the hosts from which fresh viruliferous juices were obtained for use in these studies. Dilution was with sterile water and the dilution made was used immediately as material for inoculation.

TABLE 3.—*Data from experiments to show the effect of heating viruliferous plant juices for 10 minutes at different temperatures on disease production by the southern celery-mosaic virus*

Source of inoculum	No. tobacco plants infected out of 5 inoculated								
	Temperature treatments (° C.)								
	20–24	45	50	55	60	65	70	75	80
Tobacco (Broad leaf)	5	5	5	5	4	0	2	0	–
" "	5	5	5	5	0	0	0	0	–
" "	5	–	5	–	5	–	1	0	–
Tobacco (Turkish)	5	–	–	4	–	2	–	1	0
" "	5	–	–	5	–	0	–	1	0
Cucumber (White Spine)	5	5	5	3	2	0	0	0	–
" "	5	5	5	3	1	0	0	1	–
Cucumber (Early Fortune)	5	–	–	5	–	2	–	1	0
" "	5	–	–	2	–	1	–	0	0
Celery (Golden Self Blanching)	5	5	4	4	0	0	0	0	–
" "	5	5	2	4	1	0	0	0	–
" "	5	–	2	–	1	0	0	0	–
" "	5	4	1	2	0	1	0	0	0
Celery (Special)	5	5	3	–	1	–	0	–	0
" "	5	5	1	–	1	–	0	–	0

Juice from young diseased cucumber seedlings when diluted 1:10,000 was infective, a total of 3 to 5 lesions being produced on a series of 10 inoculated cucumber cotyledons. Only an occasional lesion was produced on series of 10 cotyledons inoculated with juice diluted 1:100,000 and some series were entirely free from infection. Viruliferous extract from young tobacco plants was apparently less infective but one or two lesions occurred when 10 cucumber cotyledons were inoculated with juice diluted 1:10,000. Juice from diseased celery seedlings selected at random was erratic in behavior. The highest dilution of this material that gave infection was one of 1:1,000. The sap of *Commelina nudiflora* is viscous and the problem of diluting the juice of this host is being studied further. In the preliminary experiments made it was found that when viruliferous Commelina juice is diluted 1:500 or more no infection could be obtained when it was used as inoculum.

It is believed these experiments point out that data from dilution studies are complicated and are not good criteria to determine a virus characteristic, except, for instance, as they may indicate the freedom with which the virus has multiplied within a given host plant grown under given conditions.

Tolerance to Aging in Vitro. It is questionable just what factors may be involved in causing death of the virus when it is stored *in vitro* in unsterilized

and unfiltered from diseased plants. Though the data differ somewhat in separate experiments under a wide range of conditions it is apparent that the life of the virus of southern celery mosaic is fairly short when stored in plant juices. Juice from diseased cucumber seedlings, allowed to stand only 4 hours at a warm temperature (28° C.), shows a considerable reduction in the infective properties of the southern celery-mosaic virus. On the other hand, it is interesting that viruliferous juice from cucumber seedlings may be kept at least 2 days at 28° and still retain some of its infectiveness. When kept at ordinary room temperature (18° to 20° C.) the juice remains infective from 6 to 8 days. When the juice is stored at 3° C. it may remain infective for 2 weeks and possibly longer, though at the close of that time considerable reduction of infectivity of the virus apparently has occurred. If the viruliferous juice is frozen immediately after being expressed it retains its active virulence apparently unhampered over a period of at least 2 weeks. It has not been kept longer than that.

Influence of Desiccation on the Virus. The southern celery-mosaic virus is not destroyed immediately upon rapid drying. Tobacco seedlings were used as test plants in these studies. Squares of cheesecloth were dipped in infective juices from tobacco and cucumber plants and then air dried in a room at 26° C. Under these conditions the juices produced the disease after 72 hours drying. After 120 hours no infection could be obtained. However, infection was seriously reduced, even after the juices had dried 12 hours, and at the close of 24 hours of drying there was a 75 per cent reduction in diseased plants resulting from inoculation. After 72 hours of drying the virus infected only occasional plants (1 diseased in 15, 2 in 15, and 1 in 30).

Upon securing the above results the possibility of carrying the virus over in dried plant material was further studied. It appears that diseased leaf lamina of celery, cucumber, and tobacco lose the virus they contained when succulent when they become dry enough to crumble into powder. Numerous tests were made of such leaves from the 3 hosts, ranging in period after drying of 1 day to 5 months. No infection ever was obtained from them. Curiously enough, however, cucumber cotyledons that had numerous local lesions retained their infectious properties 2 days after being dry enough to crumble.

Effect of Chemicals on the Virus. It has not been possible to make exhaustive study of the effect of chemicals on the southern celery-mosaic virus. If properly carried out such a study would be long and involved, but in this case, only a few easily obtained chemically pure ("C.P.") laboratory chemicals have been used.

In all cases severely diseased cucumber seedlings, pulled 6 days after cotyledonary inoculation, have been used for the source of inoculum. After the stems, leaves, and cotyledons had been cut up and macerated, the pulp

was wrapped in a double thickness of cheesecloth and the juice pressed out. This was then diluted with an equal quantity of sterile water. Chemical solutions were all prepared as double-strength solutions. Equal quantities of the previously prepared virus solution were combined with an equal quantity of double-strength chemical solution. Take as an example of the method used in the mixing technique in these chemical treatment studies the 2.5 per cent sodium chloride treatment of the virus. Five cc. of freshly expressed viruliferous juice were mixed with 5 cc. of sterile water. Ten cc. of 5 per cent sodium chloride solution were added to this solution, shaken, and the mixture regarded as a 2.5 per cent solution of sodium chloride. Solutions were all prepared in this manner and then allowed to stand for 15 minutes. Small squares of cheesecloth were then wetted with the mixture and used to wipe over cucumber cotyledons, and in some cases the leaves of tobacco seedlings also were inoculated. After inoculation, the plants were allowed to stand 3 minutes and then washed with a stream of water (*cf.* Holmes (12)) to avoid killing the plants by chemical action. In some cases a large series of plants were inoculated and half of them left without washing. In some instances, *e.g.*, 5.0 and 2.5 per cent sodium chloride solutions, the plants were killed when the cotyledons and leaves were not washed. When washed, the test plants lived and allowed the virus remaining after chemical treatment to produce primary lesions.

The data from representative chemical-treatment tests are presented in table 4. Duplicate series have given comparable results when either cucumber or tobacco seedlings were used. Consequently, results from such experiments have not been included in this table. It will be seen (Table 4) that, while 50 per cent ethyl alcohol was not completely harmless to the virus, it sufficed to cut down the resultant lesions to only half that obtained when the juice was not treated with any chemical. Solutions of 1 per cent

TABLE 4.—*The effect of certain chemicals on the southern celery-mosaic virus*

Chemical	Strength of chemical (per cent)	Total no. of virus lesions on 10 cucumber cotyledons
Ethyl alcohol (absolute)	50.00	28
Hydrochloric acid (c.p.)	0.05	14
None	.....	58
Nitric acid (c.p.)	1.00	0
Calcium hydrate (c.p. saturated solution)	50.00	0
Sodium chloride (c.p.)	10.00	0
" "	5.00	2
" "	2.50	19
None	.....	56

nitric acid, a 50 per cent saturated hydrated lime solution, and 10 per cent sodium chloride all acted as perfect inhibitants of virus infection when the otherwise infective juice was treated for 15 minutes. Treatments with weaker strengths of sodium chloride solution were made. A 5 per cent solution was apparently close to the critical point at which the virus would no longer be infective, since only 1 to 5 lesions developed; whereas untreated juices produced an average of about 60 lesions. A 2.5 per cent sodium chloride solution was less effective as an inhibitant than the 5 per cent mixture. It is interesting to note that a .05 per cent hydrochloric acid solution produced about the same effect on the celery virus that the 2.5 per cent sodium chloride solution did.

Studies on Cell-Inclusion Bodies

A search for any notable differential cytological phenomena characteristic of the celery-virus disease has been carried on by the writer. The specificity of forms of "cell-inclusion bodies" may at times be questioned, but Kunkel (18) has shown that they are characteristic of a certain corn mosaic; Rawlins and Johnson (22) and Hoggan (11) show that they are commonly present in tobacco mosaic-infected solanaceous host tissues, while certain other viruses on the same hosts do not produce these curious bodies. Lately, Grant (8) has found that the ordinary tobacco virus when attacking various nonsolanaceous hosts is characterized by typical cell-inclusion bodies in the tissues.

In the present study the writer stained with Heidenhain's iron-alum haematoxylin severely affected chlorotic leaf tissues of plants affected with southern celery mosaic. However, no specifically differentiating cytological phenomena were found, and no cell-inclusion bodies of any sort appeared. The diseased plants were grown under conditions that were ideal for development of cell-inclusion bodies in ordinary tobacco-virus-infected plants, since the leaf tissues of tobacco plants, infected with ordinary tobacco mosaic, growing near-by, were found to contain such bodies. The following plants infected with the southern celery-mosaic virus were used in this study: celery, *Apium graveolens* L., cucumber, *Cucumis sativus* L., Broadleaf tobacco and Turkish tobacco, *Nicotiana tabacum* L., zinnia, *Zinnia elegans* Jacq., French marigold, *Tagetes patula* L., red garden beet, *Beta vulgaris* L., California bluebell, *Phacelia whitlavia* Gray, and Flora's paint brush, *Emilia sagittata* DC.

The affected portions of leaf tissues were considerably reduced in thickness, which was especially notable in the much shortened palisade cells and more compact spongy parenchyma. The numbers of chloroplasts appeared to be about the same as in healthy cells but they were very small and suppressed in development. Also the vascular strands were smaller in diame-

ter than comparable healthy tissues. But neither the so-called "x-bodies," "striate material," nor any other cell-inclusion peculiarity was observed in the tissues of any species of plants infected with southern celery-mosaic virus.

Studies on Rate of Virus Spread

Under the summer growing conditions in the greenhouse at Arlington, Virginia, systemic infection by southern celery-mosaic and common cucumber-mosaic viruses in tobacco, tomato, and cucumber plants first appeared in those inoculated with the celery virus. The difference in time of symptom appearance was generally from 1 to 3 days but often 4. Sometimes this difference was not so marked in cucumber plants, especially young seedlings, but careful scrutiny seemed always to decide the rate of appearance of systemic infection slightly in favor of the celery virus.

During a few weeks' stay at the Boyce Thompson Institute, a study was made with F. O. Holmes to see if infection with the southern celery-mosaic virus might produce an iodine staining pattern similar to that obtained by him (13) with ordinary tobacco mosaic and to see if this method might lend itself to other investigations of the southern celery mosaic. Although exacting in time consumed, this simple technique gives an especially valuable picture of the mode of virus spread. It was found at the outset that starch-retention patterns were produced in Turkish tobacco leaves when inoculated with the virus of both southern celery and common cucumber mosaic, incubated and stained according to Holmes's directions. Comparable series were prepared in which starch-retention patterns of common cucumber-mosaic virus, ordinary tobacco-mosaic virus and southern celery-mosaic virus on Turkish tobacco and *Nicotiana glutinosa* were studied. The treated and stained leaves were pressed and preserved and used in comparative measurements and observations. Data from these experiments are presented in table 5.

Within 24 hours, after inoculating Turkish tobacco leaves, celery mosaic caused small but distinct iodine-staining lesions that averaged 1.4 mm. in diameter. No lesions were observable in leaves inoculated either with common cucumber or ordinary tobacco mosaic. Thirty hours later the cucumber-virus lesions were just appearing and were about .3 mm. in diameter in affected leaves. Still there was no observable symptom of tobacco-virus infection. At the close of the 48-hour period after inoculation, the tobacco virus had caused lesions that were about half the size of celery-virus lesions noted 24 hours previously. Once tissue infection was established, the 3 viruses appeared to progress at considerably different rates.

In all 3 cases a certain amount of increase in area of affected tissue occurred about the original points of primary infection. The diameter of the

TABLE 5.—*A comparison of the time of production and progress of starch-retention patterns produced by the viruses of ordinary tobacco, common cucumber, and southern celery mosaic in Turkish tobacco plants*[a]

Hours[b] after inoculation	Virus used	Diameter[c] of primary lesions mm.	Systemic spread
24	Tobacco	none apparent	.....
24	Cucumber	" "	.....
24	Celery	1.4	none
30	Tobacco	none apparent	.....
30	Cucumber	0.3	none
30	Celery	2.0	"
48	Tobacco	0.75	none
48	Cucumber	2.0	"
48	Celery	4.0	"
60	Tobacco	.....	.....
60	Cucumber	2.6	none
60	Celery	4.1	very slight
72	Tobacco	2.0	none
72	Cucumber	4.7	started
72	Celery	6.2 (indefinite edges)	in old and young leaves
96	Tobacco	2.7	in young leaves
96	Cucumber	6.3 (indefinite edges)	in old and young leaves
96	Celery	8.2 (some coalesced)	in old and young leaves
168	Tobacco	coalesced	in young leaves
168	Cucumber	"	in old and young leaves
168	Celery	"	in old and young leaves

[a] These data are the result of series of studies made by means of the Holmes technique (13) for detection of starch-retention patterns due to virus infection.

[b] Hours elapsed after inoculating leaves and placing plant in dark, cold (10° C.) room and later in dark, warm (22° C.) closet, in accordance with practice outlined by Holmes (13).

[c] After bleaching of chlorophyll and staining of starch, plants were dried and preserved. Diameter of lesions represents the average measurement of 10 lesions on each of the 3 leaves containing the largest number of lesions.

southern celery-virus lesions increased to over 3 times that of the ordinary tobacco-virus lesions. Measurements showed that celery-virus lesions also were slightly more than $\frac{1}{3}$ larger than those of the common cucumber virus. These diameter differentials were consistent and typical.

First indications that the ordinary tobacco virus was escaping into surrounding tissues from the primary lesions was not noticeable until nearly 96 hours after inoculation. With the southern celery-mosaic virus, how-

ever, secondary spread was well started 60 hours after inoculation. It was not until nearly 72 hours after inoculation that the common cucumber virus had started its systemic spread. A more detailed analysis showed that after the first appearance of primary lesions systemic infection occurred in these experiments with celery virus in about 36 hours, with cucumber virus in about 42 hours, and with tobacco virus in about 66 hours.

Seventy-two hours after inoculation the greatest differences were notable in the action of the 3 viruses on young Turkish tobacco plants. Tobacco-virus lesions averaged 2.0 mm. in diameter, the disease was not systemic, and the plants appeared perfectly normal. Cucumber-virus lesions averaged 4.7 mm. in diameter, and iodine staining showed that systemic spread had commenced, though living plants showed no surface signs of disease. Celery mosaic-virus lesions averaged 6.2 mm. in diameter, systemic spread of the virus had gone through the plant, causing numerous distinct, chlorotic, vein-clearing patterns in the younger leaves, the plants were slightly stunted, and invaded leaves had taken on a crumpled and more than ordinarily pubescent appearance.

The results just reported for the southern celery and common cucumber viruses on Turkish tobacco were found to be essentially identical with those from similar studies made on *Nicotiana glutinosa*. This host also developed large whitish, canescent lesions at points of primary infection by the celery virus, becoming somewhat necrotic at about the time vein-clearing symptoms were well advanced. Besides being slower in producing vein-clearing symptoms, the cucumber virus did not produce observable primary lesions. As usual, ordinary tobacco virus produced typical small, dark necrotic lesions but no systemic infection.

Subsequent to the above observations on comparative time of appearance of infection and rate of spread of viruses in *Nicotiana* species, White Spine cucumber cotyledons were inoculated with typical celery, cucumber, tobacco, and tobacco-ring-spot viruses. This was done by wiping, as described in the foregoing. These experiments were repeated several times, and it was found that round, primary lesions developed from typical cucumber, celery, and ring-spot-virus inoculations, but no infections of any sort resulted from tobacco-virus inoculation. Exact time of appearance of lesions on cotyledons varied slightly. Generally speaking, it took from 3 to 4 days, though at times the celery virus caused primary lesions to appear towards the close of a 2-day period. Cucumber-virus lesions always appeared after celery-virus lesions, and ring-spot lesions developed last.

Southern celery-mosaic lesions on cucumber cotyledons have been described. Typical common cucumber-virus lesions are a little more marked in appearance than those produced by the southern celery-mosaic virus, producing larger indentations in the tissues on the under surface of cotyle-

dons. Tobacco-ring-spot lesions are much less marked, apparently not necrotic, and do not cause rapid collapse and drooping of cotyledons, as do the other 2 viruses. In one series of experiments measurements of lesions were made. With the celery virus the initial diameter averaged 1.3 mm., cucumber-virus lesions averaged 3.1 mm., and ring-spot lesions averaged about 3.2 mm.

Following cotyledonary inoculation of young cucumber seedlings with the typical ring-spot, celery and cucumber viruses, often the first symptoms to appear in the true leaves are numerous rounded chlorotic spots. Judging from time of appearance of such symptoms, cucumber and celery viruses become systemic within a day after primary lesions develop on the cotyledons. Ring-spot, on the other hand, remains more or less localized for from 2 to 3 days or even longer, before becoming systemic. It is also common during periods of high temperature for the celery virus to cause a watersoaked appearance of cucumber seedling hypocotyls followed by prostration and death of plants within a few days after inoculation. Under the same conditions this occurs also when seedlings are infected with the cucumber virus, but it usually becomes evident some time after the celery-virus-infected seedlings have collapsed. Such seedling collapse has not been observed in the writer's experimentations with the ring-spot carried on in parallel series with the cucumber and the celery viruses.

HOST-RANGE STUDIES

It already has been pointed out by Doolittle and Wellman (5) that southern celery mosaic is a typical virus disease of the mosaic type, is transmissible by artificial juice inoculations as well as by using the common melon or cotton aphid (*Aphis gossypii* Glov.), and is similar to common cucumber mosaic in its symptoms on certain hosts. In paralel series of experiments similarity of symptoms on cucumber seedlings of southern celery mosaic, common cucumber mosaic, and tobacco-ring-spot mosaic has just been pointed out. At times, symptoms of ordinary tobacco mosaic on Broadleaf tobacco and southern celery mosaic on the same host have marked resemblance. During the study of the southern celery-mosaic virus it was even considered possible that it might be the latent "healthy"-potato or tobacco-mottle virus. Host-range studies were instituted to determine relationships to these other viruses. Numerous series of plants of known susceptibility and reaction to definite viruses were grown and inoculated with southern celery mosaic and parallel series were inoculated at the same time and in identical manner with viruses with which comparison was desired.

Legumes

Certain legumes were studied: Tennessee Green Pod, Golden Cluster, Red Valentine, Kentucky Wonder, White Marrow, Giant Stringless Green Pod, Improved Round Pod Valentine, and Refugee varieties of the common garden bean (*Phaseolus vulgaris* L.), and in addition, two other legumes, the Broad bean (*Vicia faba* L.) and the Black cowpea (*Vigna sinensis* Endl.). The garden beans were inoculated by the wiping method at different ages on both adult and juvenile leaves, and reinoculated once and in one series 3 times. No symptoms of celery-virus infection were found on any of the garden beans. Juices obtained from over a hundred plants, representing inoculated individuals of every variety, were used to inoculate cucumber cotyledons but no virus lesions or systemic infection resulted.

The Golden Cluster garden bean apparently was immune from the celery virus, though ordinary tobacco virus caused numerous typical primary lesions, such as have been described by Price (20). The Black cowpea was not infected by the celery virus and was not a symptomless carrier of the disease, since many inoculated plants were used as the source of inoculum but no virus was recovered. A parallel series inoculated with the tobacco-ring-spot virus developed primary lesions and systemic infection typical of the disease as described by Price (21). *Vicia faba* developed small purplish primary lesions when inoculated with celery virus, but the disease did not become systemic.

Celery

Mosaic symptoms on celery (Golden Self Blanching) in the field in Florida have been described by Foster and Weber (7) and discussed in more detail by Doolittle and Wellman (5). In the field the most striking symptoms are a yellowed condition of the foliage and apparent stunting of the plant (Fig. 1, A). On close inspection, irregular areas of pale green and yellowish color are found in leaf lamina similar to mosaic diseases on other hosts. These patterns often are brilliant, developing a mottled appearance that is quite characteristic of the disease. This is illustrated in figure 3, and is notable at times for its watered moiré-like effect. There is also sharp geotropic curvature of fleshy petioles and rachises of the compound leaves. These symptoms often are accompanied by buff, shrivelled, and sunken areas of these portions of the leaves (Fig. 1, A). Southern celery mosaic may cause some twisting and savoying of leaflets but apparently does not produce filiformities or other marked distortions.

Seedling celery plants, infected side by side with the southern celery-mosaic virus and the common cucumber-mosaic virus, differed somewhat in systemic symptoms, since (Fig. 4) the mottling produced was more brilliant in southern celery mosaic. At an early stage of infection, plants infected

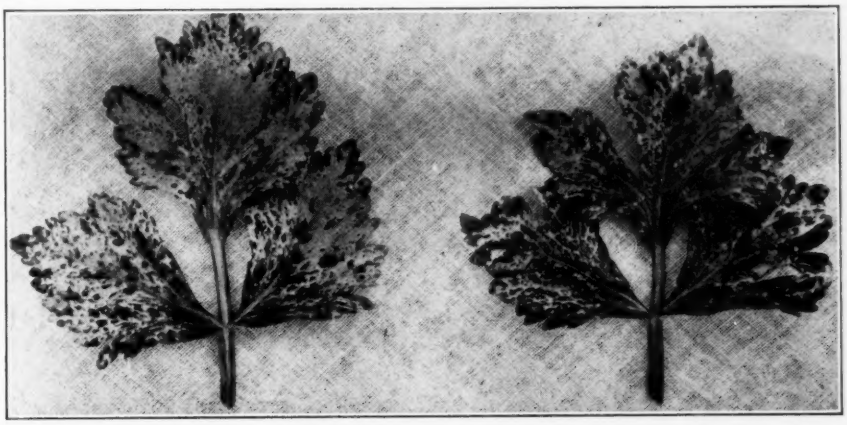

FIG. 3. Leaflets of nearly mature Golden Self Blanching celery plant, which show brilliant patterns of watered type characteristic of the southern celery mosaic. These leaflets came from a naturally infected plant growing near the spot where, 3 seasons before, the plant was obtained that furnished the original source of the southern celery-mosaic virus used in the virus-identification studies here reported. Compare with figure 5, B.

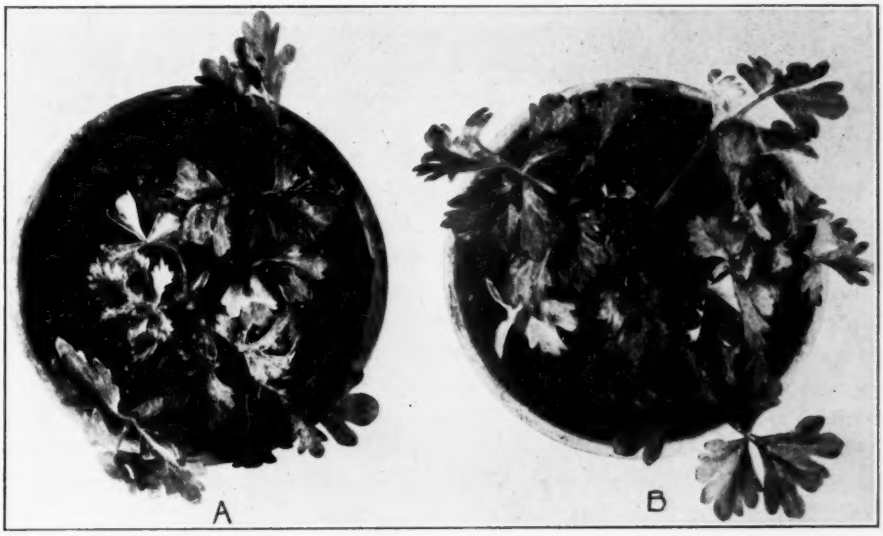

FIG. 4. Golden Self Blanching celery seedlings inoculated 9 days before the photograph was taken, showing the first systemic symptoms of infection by the virus of southern celery mosaic and the virus of common cucumber mosaic. A. First symptoms of southern celery mosaic, consisting of bright vein clearing and the beginning of a bright mottling pattern, and a downward curvature of leaf rachises and petioles that gives a stunted appearance to the plant. B. First symptoms of infection with the common cucumber-mosaic virus producing much milder mottling and vein-clearing patterns on the leaves, with less curvature of petioles and rachises.

with common cucumber mosaic and tobacco ring-spot showed symptoms similar to those of southern celery mosaic, except that chlorotic spots on leaflets and curvature of petioles and rachises were most marked in plants affected with the southern celery-mosaic virus. Leaves, on plants infected with common cucumber mosaic, after about a month, showed filiformities and raised blister-like areas following a mild mottling (Fig. 5, B). These leaves, moreover, became dark green and upright in growth habit rather than chlorotic and curved downwards, as in the case of southern celery mosaic. These symptoms produced by the common cucumber virus compare well with those described by Poole on celery; and the writer is convinced that Poole (19), Harvey (9), and Elmer (6) were working with common cucumber mosaic (*Cucumber virus 1*) on celery. Plants infected with tobacco-ring-spot virus for a month exhibited a pattern of small, green and yellowish spots, many of which contained small necrotic areas. Leaflets on these plants were also malformed, producing "shoe-string" effects, and the whole plant was more yellow than those infected with either the cucumber or celery mosaic.

Cucurbits

Systemic symptoms of 3 mosaics have been studied on White Spine cucumber seedlings. Under certain environmental conditions as yet not fully understood, seedlings diseased with southern celery mosaic may not develop the hypocotyl collapse, prostration, and final death of the plant, such as has already been noted. If conditions at this critical period are such that individuals do continue growth, systemic mottling develops, and it is quite different in appearance from that produced by the viruses of the common cucumber or tobacco-ring-spot mosaics.

Cucumber seedlings, infected with southern celery mosaic, and that have survived the time when they usually die, are markedly stunted, the hypocotyls are so weak as to be unable to support the plants in a normal upright position, and the leaves display (Fig. 5) an indistinctly marbled or irregular moiré-like pattern, roughly suggestive of a dull yellow and green watered figure on cloth. The marked angular areas and more general chlorosis of true cucumber mosaic (*cf.* Doolittle (2)), and the definitely round-spotted effect accompanied by slight necrosis of tobacco-ring-spot mosaic (*cf.* Wingard (24)) are not the same as the southern celery-mosaic symptoms. Differentiation of these viruses, however, based on systemic symptoms on cucumber seedlings is often deceiving, since there are times when the symptom effects grade into one another most confusingly.

It should be noted, in connection with cucurbitaceous hosts, that southern celery mosaic is capable of readily infecting both Cocozelle and Summer Crookneck squashes, and also muskmelon and watermelon seedlings. In the

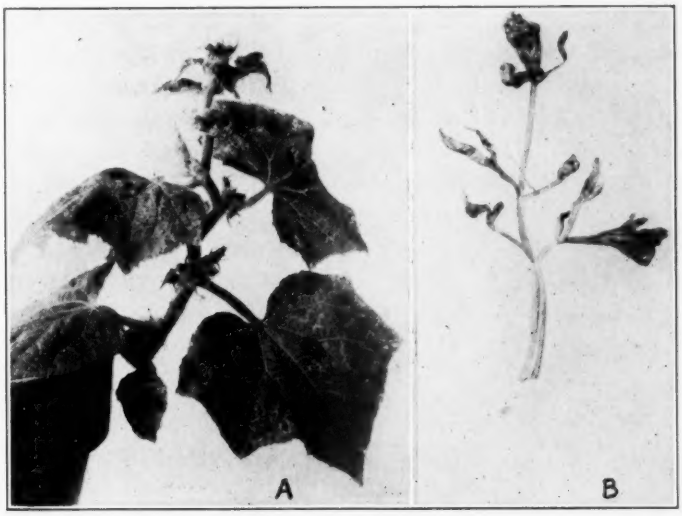

FIG. 5. Systemic symptoms of southern celery mosaic on cucumber and common cucumber mosaic on celery. A. Cucumber (White Spine) plant systemically infected with southern celery-mosaic virus for about 3 weeks. Note characteristic marbled or moiré-like mottled pattern on the leaves, and shortening of internodes. B. Leaf of celery (Golden Self Blanching) plant systemically infected with common cucumber mosaic virus for about six weeks. Note severe malformation of leaflets, which includes twisting, blister-like effects, filiformities, and thickened lamina. Compare the appearance of this leaf with figures 1, A and 3.

case of watermelons (Kleckley Sweet, Tom Watson, and Florida Favorite varieties were used) their susceptibility was especially interesting, since, according to Doolittle and Walker (4) as well as the writer, these hosts apparently are immune from common cucumber mosaic.

Emilia

A flowering plant, Flora's paint brush (*Emilia sagittata*), was found to be susceptible to ordinary tobacco (*cf.* Grant (8)), and the common cucumber and southern celery-mosaic viruses. All 3 viruses, used as inoculum under summer conditions at Arlington, Virginia, infected this host systemically and could be easily recovered from the infected leaves. The celery virus, however, produced no visible symptoms, while tobacco-mosaic-infected plants were severely mottled, and the cucumber virus caused a mild mottling.

Datura

In studying a possible relationship of southern celery-mosaic to the latent potato- or tobacco-mottle virus described by Johnson (14), parallel series of Jimson weed (*Datura stramonium* L.) seedlings were inoculated

with juice from celery infected with the southern celery-mosaic virus and with juice from potatoes infected with the tobacco-mottle virus. The plant showed a typical reaction to the tobacco-mottle virus resulting in characteristic reticulate patterns on leaves systemically infected. Inoculated leaves of Jimson weed plants, used to test the southern celery-mosaic virus, began dropping about a month after inoculation. They were characterized at that time by a few large, diffuse, whitish spots at points of infection. It was possible to recover the southern celery-mosaic virus from such leaves, but repeated trials of plant parts that had not been inoculated gave negative results.

Peppers

Several varieties of garden peppers inoculated by the wiping method with southern celery-mosaic virus were all susceptible to it. Small seedlings of Tabasco pepper (*Capsicum annuum* var. *conoides*) developed black primary lesions on leaves inoculated with the celery virus within a period of 3 to 8 days. Moreover, the disease became systemic almost as soon as the lesions appeared, becoming first noticeable as a twisting or crook neck of the tender tip region of the stem. After a few days the crook straightened out; the plant remained living but severely stunted and marked mottling symptoms had developed on leaves systemically infected while young. When inoculated with the cucumber virus no infection was obtained. These plants were reinoculated, kept for a week, and the juice used as inoculum on cucumber seedlings; but, apparently, no common cucumber-mosaic virus was present.

Plants of a variety of sweet pepper (*Capsicum frutescens* var. *grossum*), California Wonder, were inoculated by wiping with the common cucumber-, southern celery- and ordinary tobacco-mosaic viruses. In series of 10 plants each the tobacco virus caused 10 to become diseased, the cucumber virus infected 7, and the celery virus infected 6. The ordinary tobacco virus was more rapid in its infection than the celery virus, producing systemic infection 3 to 7 days after inoculation. A typical black streak symptom developed on tender stems, and leaf petioles adjacent to streak marks on stems yellowed and dropped almost immediately after its appearance. The southern celery-mosaic and common cucumber-mosaic viruses were systemic 9 days after inoculation. With the celery virus a typical light brown streak appeared on sweet-pepper seedlings, causing as severe dropping of leaves as did the tobacco virus, and sometimes killed seedlings. Many plants also had the crook-neck symptom similar to the celery virus attack on Tabasco pepper. These plants soon straightened up and, although the plants were not always killed, the flowers dropped or did not mature fruits. Systemic mottling caused by the tobacco virus was severe, as was

mottling due to the celery virus. The cucumber-virus mottling apparently was of a milder type, though badly affected plants did not produce fruits. Stunting of growth was characteristic of the disease produced by all 3 viruses, though it varied somewhat in intensity, depending on point of inoculation.

Commelina

Commelina nudiflora has already been named and discussed as a host for southern celery mosaic (3, 5, 23) and is illustrated in figure 1, B. This host seems to be immune from infection by *Tobacco virus 1.* Thus far, attempts to inoculate it with the common cucumber-mosaic virus also have failed. The southern celery-mosaic virus does not infect Commelina when inoculations are made by the rubbing method. It appears to require insect transmission or the use of needle or hypodermic methods of inoculation to produce infection. The southern celery-mosaic virus, once it becomes systemic, exists from year to year without much effect on the normal behavior of the plant. Systemic infection is the rule; the mosaic patterns on leaves consist of yellowish green areas that are not angular but give a mildly marbled or watered and moiré-like appearance. It is only in exceptional cases that leaf malformations have been found, and there is apparently no death of tissues (Fig. 1, B.).

Tomato

Tomato plants (Globe), growing side by side and inoculated with the southern celery-mosaic, common cucumber-mosaic and ordinary tobacco-mosaic, exhibited symptom differences. The ease of infecting tomato seedlings by ordinary tobacco mosaic is known by students of this virus. These plants showed severe systemic infection with the ordinary tobacco mosaic within 3 to 4 days after inoculation. Of 10 plants inoculated, 10 were diseased. Under the same conditions, it took about 9 or 10 days for systemic infection (vein clearing) to appear on tomatoes inoculated with southern celery-mosaic and, of 10 inoculated, only 2 were diseased. The common cucumber-mosaic virus caused vein-clearing symptoms at about the same time as did the celery virus, and, of 10 plants inoculated, 4 were diseased.

The 3 series of plants were examined 30 days after inoculation. Ordinary tobacco-mosaic-infected tomatoes were badly stunted, the leaves were crumpled and much distorted and somewhat blistered, and they showed characteristic yellow and green mosaic mottling, typical of the disease. In some cases apparently complete inhibition of flower development occurred, and the flower buds did not open. Plants systemically infected with southern celery mosaic were less stunted than those affected by the tobacco mosaic. The leaves were marked with a reticulate pattern and mild

mottling but there was no distortion of leaf lamina. The flowers were few in number but appeared to be normal. The common cucumber-mosaic-infected plants were more stunted than those affected with the celery-mosaic virus, but not so severely shortened as ordinary tobacco-virus-infected individuals. However, plants infected with common cucumber-mosaic virus were distinctly characterized by much filiformity of leaves, accompanied with little mottling, and, although flowers were present, they were weak and dropping.

Nicotiana Species

Plants of the genus Nicotiana were used as possible hosts to differentiate the celery, cucumber, and tobacco viruses. It has been demonstrated by Holmes (12) and others that *N. glutinosa*, when inoculated with ordinary tobacco-mosaic virus, develops typical small black lesions of diagnostic value. Parallel series of plants of this species were, therefore, inoculated by the wiping method with the common cucumber-, the southern celery-, and the ordinary tobacco-mosaic viruses. Typical lesions of ordinary tobacco virus appeared 2 days after inoculation with this virus but they did not enlarge greatly and the virus remained localized. The first symptom of southern celery-mosaic infection appeared on the plants 6 days after inoculation as numerous whitish, round spots, about 5 mm. in diameter on the inoculated leaves. The spots were not necrotic but became even whiter during the next few days. Such canescent spots were stained with iodine, as described by Holmes (13), and found to be primary lesions at a late stage of development. The first vein-clearing symptoms appeared on plants 7 days after inoculation and, a day later, systemically infected leaves were more yellowed and had a somewhat blanched appearance. Four days after, vein-clearing, necrotic, zigzag lines started to develop on these leaves and primary lesions also began to be necrotic. Plants inoculated with common cucumber mosaic did not produce canescent primary lesions, and appearance of vein clearing occurred a day later than with the celery virus. Systemic mottling and chlorosis were not so serious on the cucumber-virus-infected plants and no necrotic effects developed.

Symptoms on Turkish and Broadlef tobacco (*Nicotiana tabacum*) seem to be almost identical when infected with the common cucumber virus or with the southern celery-mosaic virus. Primary lesions of the whitish type described on *N. glutinosa* are produced by the southern celery-mosaic virus on both Turkish and Broadleaf tobacco. Old lesions of this type are illustrated in figure 6. No primary lesions were noticed in connection with the common cucumber-virus inoculations. Upon systemic infection of the 2 tobaccos, southern celery mosaic causes vein clearing, followed by a more yellowish appearance and mild mottling of the plants, coupled with

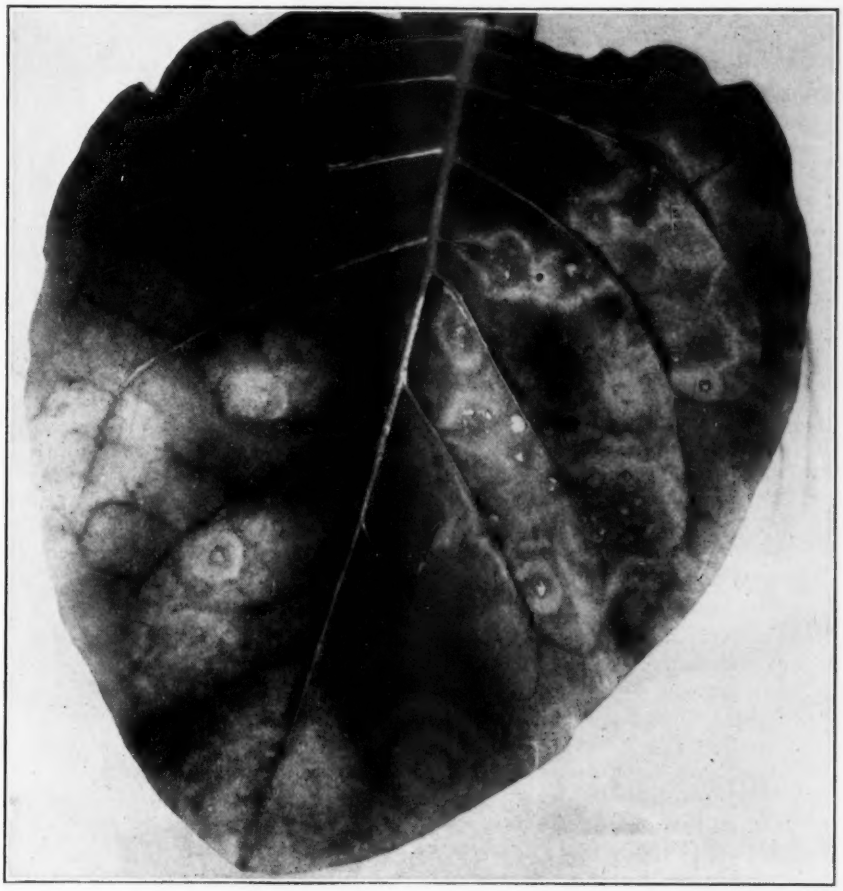

FIG. 6. A lower leaf of a Broadleaf tobacco plant, inoculated with the southern celery-mosaic virus, 10 days before this picture was taken. Virus infection has resulted in the production of numerous canescent lesions a few of which, as the infection grows older, are becoming somewhat necrotic. Note that in some lesions on the right side of the leaf chlorophyll has come back into what was once very light-colored spots.

crumpling of the leaves downward and what seems to be a greater pubescence on such leaves. These latter symptoms are quite marked in contradistinction to the upright orientation of leaves systemically infected with the common cucumber virus, which also produces a gray type of chlorosis, spotted with a few large, dark green areas. As the diseased plants become older, differences in appearance are less marked until, at the time of blossoming, it is almost impossible to see specific differences in symptoms. Old plants, especially of the Broadleaf tobacco, affected with either the celery or the cucumber virus, often resemble to a marked degree old plants affected with the ordinary tobacco mosaic.

Susceptible Host List

As has been indicated, studies leading to fairly complete data on plant species susceptible to the southern celery-mosaic virus are in progress. Thus far, however, 2 classes and 8 families of plants represented by 23 species and varieties have been listed as susceptible and are as follows:

MONOCOTYLEDONAE
- COMMELINACEAE
  - *Commelina nudiflora* (creeping day flower or wild wandering jew)

DICOTYLEDONEAE
- CHENOPODIACEAE
  - *Beta vulgaris* (red garden beet)
- LEGUMINOSAE
  - *Vicia faba* (Broad bean)
  - *Vigna sinensis* (Black cowpea)
- UMBELLIFERAE
  - *Apium graveolens* (celery)
- HYDROPHYLLACEAE
  - *Phacelia whitlavia* (California bluebell)
- SOLANACEAE
  - *Nicotiana glutinosa* L.
  - *N. tabacum* (Broadleaf tobacco)
  - *N. tabacum* (Turkish tobacco)
  - *Datura stramonium* (Jimson weed)
  - *Lycopersicum esculentum* Mill. (Globe tomato)
  - *Capsicum annuum conoides* Irish (Tabasco pepper)
  - *C. annuum grossum* Sendt. (sweet pepper)
  - *Physalis pubescens* L. (common ground cherry)
  - *P. lagascae* R. & S. (wild ground cherry)
- CUCURBITACEAE
  - *Cucurbita pepo* var. *condensa* Bailey (Cocozelle squash)
  - *C. pepo* var. *condensa* (Summer Crook-neck squash)
  - *Cucumis sativus* (White Spine cucumber)
  - *C. melo* (muskmelon)
  - *Citrullus vulgaris* Schrad. (watermelon)
- COMPOSITAE
  - *Zinnia elegans* (zinnia)
  - *Tagetes patula* (French marigold)
  - *Emilia sagitata* (Flora's paint brush)

DESCRIPTION OF CELERY VIRUS 1

Name: Southern celery mosaic (*Celery virus 1*)

Type: This publication, figures 1, A and B; 2, A; 3; 4, A; 5, A; 6.

Host Families: Wide range, families so far listed include Commelinaceae, Chenopodiaceae, Leguminosae, Umbelliferae, Hydrophyllaceae, Solanaceae, Cucurbitaceae, and Compositae.

Differential Hosts:

Apium graveolens (Golden Self Blanching celery), severe chlorosis; downward curvature of rachises and petioles; discoloration and

shrivelling of rachises and petioles; leaflets not reduced in number, no filiformity nor upright orientation.

Cucumis sativus (White Spine cucumber), sunken lesions on underside of cotyledons; collapse of hypocotyl and death of seedlings more rapid than with *Cucumber virus 1;* watered, moiré-like pattern on leaves of seedlings escaping death.

Datura stramonium (Jimson weed), diffuse primary lesions on leaves; not systemic; infected leaves drop.

Capsicum annuum var. *conoides* (Tabasco pepper) black primary lesions; necrosis of stem and crook neck; stunting and mottle.

Commelina nudiflora, no primary lesions; systemic mottling of moiré-like pattern; no necrosis.

Lycopersicum esculentum (Globe tomato), difficult to infect; mottling mild and may be reticulate pattern; no severe distortion of leaf lamina.

Nicotiana glutinosa, distinct, canescent primary lesions, becoming necrotic; yellowish blanched systemic symptoms; necrotic, zigzag patterns on systemically infected leaves.

Nicotiana tabacum (Broadleaf tobacco), canescent primary lesions becoming necrotic; brilliant vein clearing; chlorosis and downward crumpling of leaves; more than ordinarily pubescent appearance; and zigzag necrotic patterns.

Resistance to Aging *in vitro:* 6 to 8 days at room temperature.

Resistance to Desiccation: 72 hours on cheesecloth, killed when leaf tissues are dry enough to crumble.

Thermal Death Point: 75° C., 10 minutes.

Resistance to Chemicals (15 minutes): resists 50% C_2H_5OH; killed by .02% HNO_3, .1% NaCl, and 50% sat. sol. $Ca(OH)_2$.

Resistance to Dilution: variable, maximum 1:100,000.

Cytological Characters: no inclusion bodies; suppressed chloroplast development; shortened palisade cells; compact parenchyma.

DISCUSSION AND CONCLUSIONS

The studies herein reported adequately establish the identity of the virus causing the southern celery mosaic. Besides causing a disease that results in increasingly serious losses in celery, the virus has been observed by the writer in the field as very destructive on other truck crops, *e.g.,* cucumbers, Cocozelle squash, summer crook-neck squash, and sweet or bell peppers. The host range is wide and is still being studied, and, in addition to hosts mentioned in this paper, the virus is able to infect numerous other weeds, ornamentals, and truck-crop plants.

The writer has always found, in Florida, that, when plants are affected in the field with the southern celery-mosaic virus, the disease is traceable largely to the presence of a single weed, *Commelina nudiflora,* called creeping day flower or wild wandering jew. In some cases, for instance isolated fields of diseased peppers, it has not been easy to follow the connection, but always, when locations of seed beds were known and detailed surveys

of field edges were made, mosaic infection of crops could be traced to natural insect inoculation from the weed host. Yet, it should be remembered that other plants than *C. nudiflora* may act as reservoirs of the disease. It has been pointed out by Doolittle and Wellman (5) that celery with systemic symptoms apparently identical with southern celery mosaic were found in plants from Ohio, Wisconsin, New York, and California. Notwithstanding the fact that it has been reported in northern States, it is my opinion that southern celery mosaic is of subtropical or tropical origin and is primarily a disease of *C. nudiflora* that does not withstand severe spells of cold weather. This belief early led the writer to consider naming the celery-mosaic trouble, just described, Commelina mosaic (*Commelina virus 1*). Had this been done the economic side of the subject, its attack on an important crop plant, might have been obscured in later studies and it is too important to be lost sight of.

Celery virus 1 is not the same as the tobacco ring-spot studied in detail by Henderson and Wingard (10), although the properties of these 2 viruses somewhat resemble each other. The southern celery-mosaic virus will infect tomatoes when inoculated by the wiping method, whereas tobacco ring-spot apparently is incapable of this. On the other hand, the Black cowpea is immune from the southern celery-mosaic virus and tobacco ring-spot causes primary and systemic infection. On tobacco, cucumber, and celery plants infections with the 2 viruses cause distinctly different symptoms.

The ordinary tobacco mosaic, *Tobacco virus 1,* Allard, described by Johnson (15) and Allard (1), is quite distinct from *Celery virus 1.* The celery virus withstands aging *in vitro* only a few days, whereas tobacco virus withstands it for years. The tobacco-virus thermal death point is approximately 15 to 20 degrees C. higher than that of *Celery virus 1.* Cytological studies disclosed no cell-inclusion bodies in tissues infected with southern celery-mosaic virus such as those characteristic of ordinary tobacco mosaic. The celery virus infects cucurbits, which are immune from *Tobacco virus 1,* and becomes systemic in *N. glutinosa* on which the tobacco virus produces small, black, necrotic, primary lesions but no systemic infection.

Southern celery mosaic (*Celery virus 1*) is similar in some ways to cucumber mosaic (*Cucumber virus 1,* Doolittle) described by Doolittle (2), Johnson (15), and Doolittle and Walker (4) in its properties and host relationships. It may be that these 2 viruses are fairly closely related. The thermal death point of *Celery virus 1* is only about 5° C. higher than *Cucumber virus 1,* which is not a greatly significant difference. The cucumber virus withstands aging *in vitro* about 3 days, whereas the celery virus withstands the treatment 6 to 8 days. On the other hand, watermelons are immune from common cucumber mosaic but are readily infected by the southern celery mosaic. The celery virus causes nonnecrotic, whitish or

canescent, primary lesions on *Nicotiana glutinosa* and *N. tabacum*, followed by necrotic zigzag patterns upon systemic infection; but the common cucumber virus produces no such effects. Filiformities characterize leaves of celery and tomato plants infected with *Cucumber virus 1* but are not induced by *Celery virus 1*. Celery virus infection on cucumber cotyledons results in smaller primary lesions than those produced by the cucumber virus; it may cause collapse and prostration of most cucumber seedlings a few days after infection, while *Cucumber virus 1* usually causes this some days later. Cucumber seedlings that survive prostration and death due to infection with *Celery virus 1* develop systemic infection, which results in a moiré-like or watered mottling pattern in the leaves, while systemic mottling, due to common cucumber mosaic, is characterized by more angular spots and is generally more chlorotic.

Celery virus 1 is not latent-potato- or tobacco-mottle virus, for this virus, according to Koch (17), withstands 28 days' aging *in vitro*, which is 3 weeks longer than the celery virus can endure. Symptoms caused by southern celery-mosaic infection of tobacco are not like those of tobacco mottle. On *Datura stramonium*, *Celery virus 1* causes definite primary lesions, accompanied by dropping of inoculated leaves, but no systemic infection, and the tobacco mottle causes typical systemic symptoms in *D. stramonium*.

Celery virus 1 is a new virus, and is the first celery virus to have been fully described. It is believed upon careful study of symptoms described in reports of celery mosaics by Poole (19), Elmer (6), and Harvey (9) that these investigators were working with common cucumber mosaic (*Cucumber virus 1*, Doolittle). Mosaic of celery occurs in the northern celery growing areas, but ordinarily differs in symptoms from that that is the subject of this paper. For these reasons, and because the disease was so long investigated in the South and is in so many cases traceable to the presence of the subtropical and tropical weed host, *Commelina nudiflora*, it has been named "Southern celery mosaic" and the virus has been given the specific designation *Celery virus 1*.

SUMMARY

A new virus, which causes a disease of true mosaic type on celery, is here described. The virus is transmissible by *Aphis gossypii* and artificial juice inoculations. It is filterable through a bacteria-excluding Berkefeld, "W," filter. It is destroyed upon heating for 10 minutes at 75° C. Freezing does not destroy it. Viruliferous juices may be diluted with water varying in ratios from 1:500 to 1:10,000 and occasionally at 1:100,000. Such solutions still remain infective, dependent on host plants and environmental conditions. The virus withstands aging *in vitro* 2 days at 28° C.,

from 6 to 8 days at 18° to 20° C., and remains infective at least 14 days when stored in a frozen condition. It withstands drying on pieces of cloth for between 3 and 5 days, in infected leaves it is destroyed within 1 day after they are dry enough to crumble but may last 2 days in dried cucumber cotyledons. It withstands 50 per cent ethyl alcohol for 15 minutes, but 1 per cent nitric acid, 50 per cent saturated hydrated lime solution, and 10 per cent sodium chloride solutions all destroyed the virus when treated 15 minutes. Cytological studies were made of diseased leaves of several hosts, but no cell-inclusion bodies of pathological nature were found. The spread of viruses in hosts was studied and it is characteristic of the celery-mosaic virus being described that it spreads more rapidly than the ordinary tobacco-, the common cucumber-, or the tobacco ring-spot viruses.

Symptom studies of virus diseases of the mosaic type have been made on many hosts comparing ordinary tobacco mosaic, common cucumber mosaic, tobacco ring-spot mosaic, latent or healthy potato (tobacco mottle) mosaic, and the southern celery mosaic. Differences have been discovered in the symptoms of the various virus diseases produced on a number of hosts, although it is to be noted that their intergradations in many cases make differentiation on a purely symptom basis dangerous criteria of specificity of causal viruses. However, a comparative analysis by combining symptomological and property studies of the viruses discloses that the virus originally recovered from celery is new and distinct.

It has been shown that the celery-mosaic diseases reported by Poole (19), Elmer (6), and Harvey (9) probably were due to the common cucumber virus (*Cucumber virus 1*, Doolittle) attacking celery.

Host-range studies, which are not completed, show that *Celery virus 1* has caused disease in plants of 23 species and varieties representing 8 families in both the Monocotyledonae and the Dicotyledonae. In addition to serious losses on celery in the field, this virus also has been found on other truck crops, notably Cocozelle squash, summer crook-neck squash, peppers, and cucumbers.

This virus is new, was first studied on celery where it causes a destructive mosaic disease, and has as its principal wild host the subtropical and tropical weed *Commelina nudiflora*. For these reasons, the author has named the virus *Celery virus 1* and has given the disease the common name *southern celery mosaic*.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

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NONPARASITIC LEAF SPOTS OF BARLEY[1]

J. J. CHRISTENSEN

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In some years, in Minnesota, leaf spots of barley, apparently of nonparasitic origin, are more prevalent than those caused by parasites and may become severe enough on some varieties to cause complete killing of foliage and premature ripening. Velvet, Glabron, Trebi, and other varieties of barley commonly grown in Minnesota frequently develop severe spotting from the seedling to adult stage on certain kinds of soil. Local areas or entire fields may be involved. Such spotting appears to be associated with malnutrition. On most varieties, however, nonparasitic spots are perhaps of little economic importance, except insofar as they furnish an avenue of entrance to semiparasitic organisms.

Although these nonparasitic diseases have been recognized for a number of years in Minnesota, it has been difficult to obtain definite information on symptoms, since leaf spots caused directly by fungi, *i.e.*, *Helminthosporium sativum* P. K. and B., *H. teres* Sacc., and similar congeneric species, also are usually abundant on barley in this State. Furthermore, in some seasons the symptoms of nonparasitic spots may be greatly modified by secondary fungi and bacteria. It has been shown, however, that spot-blotch organisms require considerable moisture in order to produce good foliage infection (9). The growing seasons 1930 to 1933, inclusive, have been ideal for the study of nonparasitic diseases in Minnesota, because of the relative lack of precipitation. In these years, practically no spot blotch or other Helminthosporium spots developed on plots of barley containing more than 125 varieties, even when they were sprayed repeatedly with a suspension of spores of parasitic fungi. But, on these same plots, many of the varieties developed certain types of nonparasitic leaf spots.

Recently nonparasitic diseases of cereals, especially of oats, have received particular attention in Europe. In 1930, Ferdinandsen and Winge (3) described a nonparasitic leaf spot of oats, which they named heritable blotch leaf, resembling one type of lesion observed by the writer on barley. Several workers (4, 6, 8) have found that magnesium deficiency in certain types of soils may result in mottling and chlorosis of oats and barley and other crops, while others (5, 7) have reported "yellow tip" or "white tip" of cereals, which can be controlled by the application of copper sulphate to

[1] Paper No. 1217 of the Journal Series of the Minnesota Agricultural Experiment Station. The writer is indebted to Professor E. C. Stakman for helpful criticism in preparation of this manuscript.

the soil. These nutritional lesions appear, however, to be quite different from those commonly observed on barley in Minnesota.

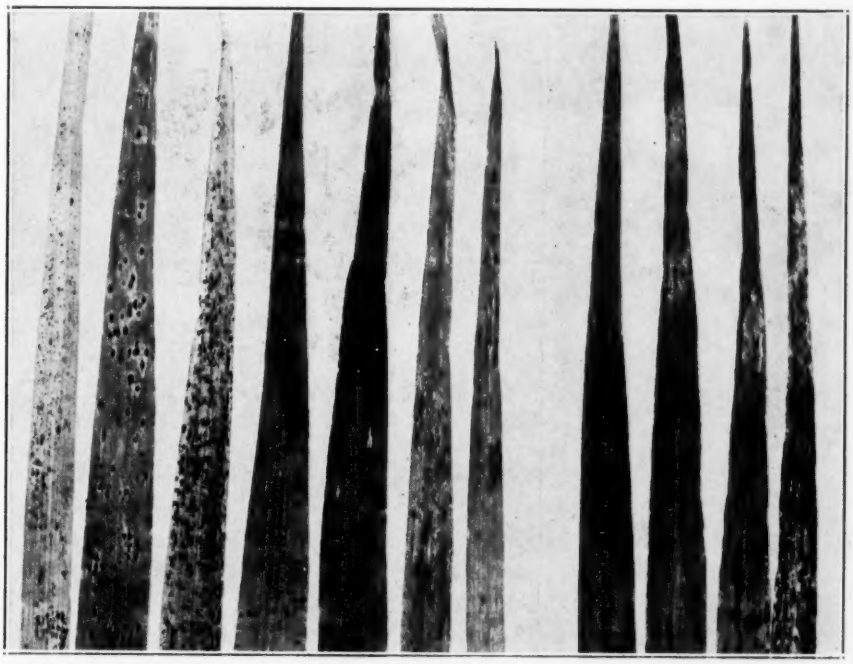


FIG. 1. Types of nonparasitic spots on different varieties of barley. A. *H. d. deficiens* selection. B. Black Hull-less C. I. 666. C. Gorak, C. I. 1086. D. C. I. 2208. E. N. S. Wales selection. F. *H. d. decorticatum* C. I. 2230. G. Lion C. I. 923. H–K. Variation in symptoms on *H. d. decorticatum*.

In some years, it is almost impossible to differentiate between true spot blotch caused by *Helminthosporium sativum* and some of the nonparasitic spots or blotches of barley, especially if the two types are found on the same variety (Figs. 1–4). Clear differentiation is of considerable importance in studies on the epidemiology of *Fungi Imperfecti*, of inheritance of reaction to leaf spot, and also in the study of varietal resistance to disease. For this reason, observations and experiments were made in the field and greenhouse on many varieties of barley[2] to obtain information on symptoms, possible causes, and methods of control of the nonparasitic leaf spots.

[2] Most of the varieties of barley used in these tests were obtained from the Division of Agronomy and Plant Genetics, University of Minnesota, and M. N. Pope, Division of Cereal Crops and Diseases, U. S. Department of Agriculture.

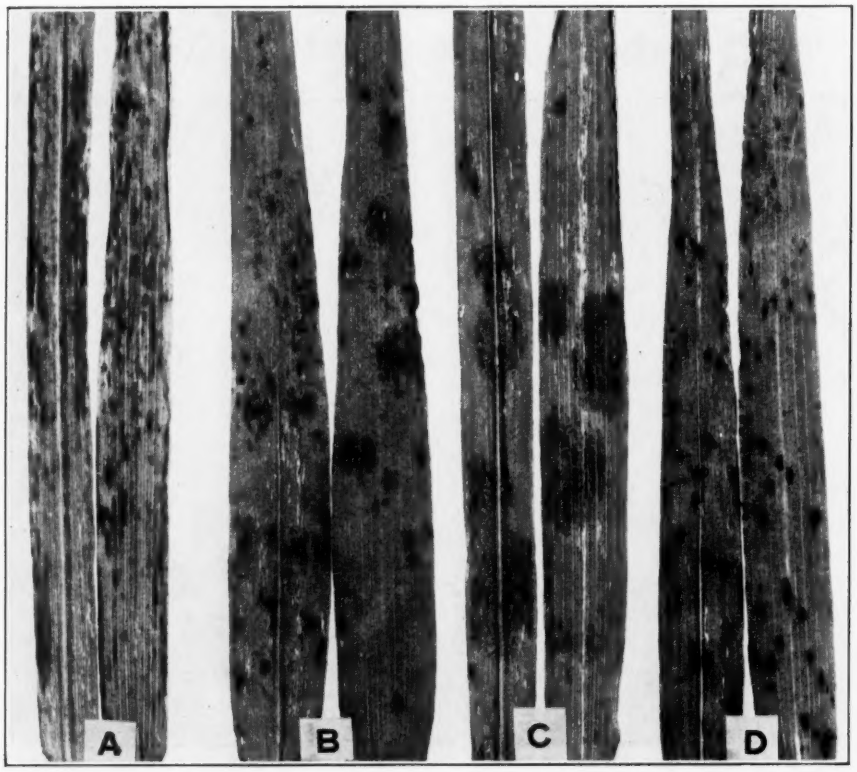


FIG. 2. Four types of nonparasitic spots on four different varieties of barley. A. Purple nudum, C. I. 1219. B. Zumpakei C. I. 692. C. *H. d. nigrilaxum* selection. D. N. S. Wales selection.

SYMPTOMS

The first evidence of the nonparasitic spots is generally the appearance of chlorotic, yellowish, or light brown areas, while the lesions produced by parasites usually begin as more or less water-soaked areas. These nonparasitic lesions can be distinctly seen by transmitted light and usually become much darker with age, varying from light brown to black. In some cases the lesions are distinct spots; in others there is a stripe effect similar to true barley stripe; and, occasionally, the entire leaf blade is bleached or discolored. There is considerable variation in depth of color under different environmental conditions, sunlight and high temperature being conducive to pigmentation. The disease area may be uniform in color throughout and sharply delimited from healthy tissue. In some varieties the outer edges of the lesion were marked by a narrow and much lighter zone (Fig. 1, B). In other cases spots were more or less diffused, so that it was almost impossible to separate diseased from healthy tissue, the effect being that of

FIG. 3. Barley plants, Yanehadaka C. I. 693, affected with leaf spots of non-parasitic origin.

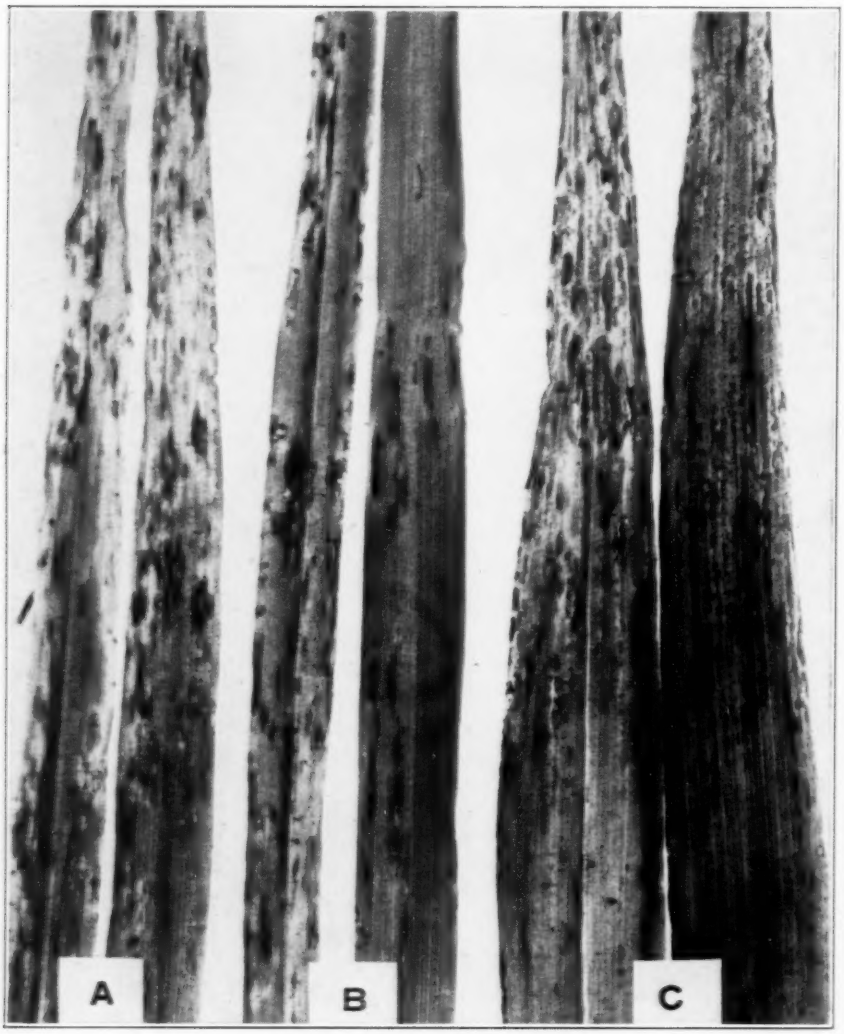


FIG. 4. Leaves from 3 varieties of barley artificially inoculated with *Helminthosporium sativum*. A. Minsturdi, C. I. 1556. B. Lion, C. I. 923. C. Black Hull-less selection.

bronzing and scalding (Fig. 2, A). The size of lesions may vary greatly, ranging from small freckle-like spots (Gyanste, C. I. 654) through all gradations to the large blotch-like lesions in such varieties as Lion C. I. 923 and Trebi C. I. 936. Sometimes two or more different types of lesions occur on the same plants (see figures 1–3 for variation in lesion on leaves).

Another type of lesion, fairly common on such varieties as Hanna C. I. 203, and Steigum C. I. 907, resembled true barley stripe caused by *Hel-*

minthosporium gramineum Rab. It was difficult sometimes to distinguish "false stripe" and true stripe, for the symptoms of the former varied from indefinite chlorotic lesions, which turn brownish with age, to the ribbon-like lesions characteristic of true stripe.

Symptoms sometimes deviate from those of the spots or the "false stripe," and the entire leaf tip is bleached or discolored, as in White Hull-less Selection. In Black Hull-less C. I. 708 the characteristic symptom is blackening of the flag leaf.

The time of appearance of the nonparasitic lesions varies with varieties. On some, such as Zunpakei C. I. 692 and *H. d. decorticatum* C. I. 2230, the lesions consistently appear in the seedling stage, although on most varieties they do not appear to a great extent until the plants have reached the jointing or boot stage. In fact, the spots usually develop most rapidly after the heading of barley.

The spotting usually, but not always, progresses from the lower to the upper leaves, the lower ones often being most severely injured. Spotting develops uniformly and simultaneously on nearly all the plants of the same variety that are in the same stage of development.

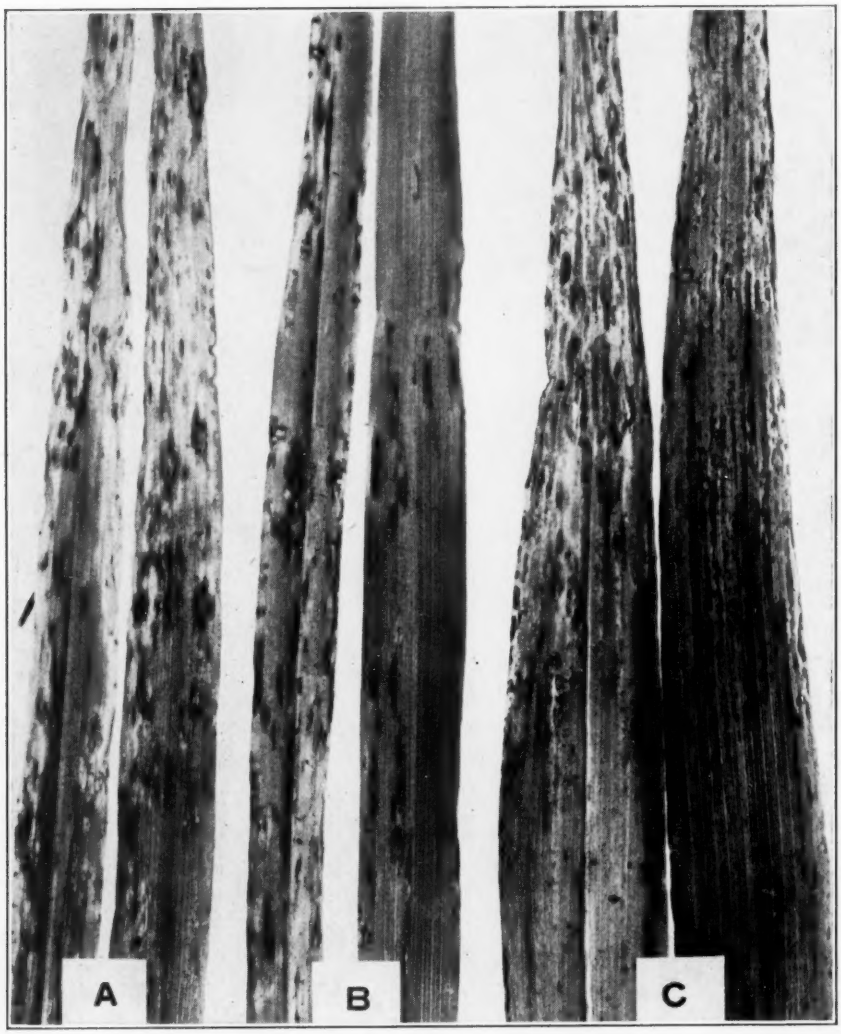
The spots may be scattered, as in the variety New South Wales Selection, or aggregated, as found in Kamet Mugi C. I. 2253. In fact, parts of leaves may be completely destroyed as a result of coalescence of numerous spots (Tatien C. I. 665), or they may become so weakened that they dry up or die from the attack of secondary organisms (Gorak C. I. 1086 and Cornutum C. I. 724). The spots produced on a given variety were similar in successive years. The characteristic symptoms, also, have been observed on many varieties when grown in the greenhouse. In most cases, however, the lesions were more distinct and larger in the field than in the greenhouse. In general, the type of spots produced is characteristic for the variety concerned, although a number of varieties may develop similar types.

EXPERIMENTS TO DETERMINE CAUSAL FACTORS

Failure to Isolate Pathogen

Numerous tests of many different sorts indicated that these leaf spots are, apparently, of nonparasitic origin and are either inherent or nutritional maladies. Many attempts were made to establish a parasitic origin but none were successful. Field and greenhouse tests indicate that healthy plants never become spotted by contact with badly spotted plants, even though their leaves and culms may be interlocking.

Attempts were made repeatedly to isolate causal organisms. Diseased leaves from many varieties grown in the field or greenhouse were plated out in the usual manner on nutrient agar. The results were always negative, except when leaves bearing old lesions were selected. From such material



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Another type of lesion, fairly common on such varieties as Hanna C. I. 203, and Steigum C. I. 907, resembled true barley stripe caused by *Hel-*

minthosporium gramineum Rab. It was difficult sometimes to distinguish "false stripe" and true stripe, for the symptoms of the former varied from indefinite chlorotic lesions, which turn brownish with age, to the ribbon-like lesions characteristic of true stripe.

Symptoms sometimes deviate from those of the spots or the "false stripe," and the entire leaf tip is bleached or discolored, as in White Hull-less Selection. In Black Hull-less C. I. 708 the characteristic symptom is blackening of the flag leaf.

The time of appearance of the nonparasitic lesions varies with varieties. On some, such as Zunpakei C. I. 692 and *H. d. decorticatum* C. I. 2230, the lesions consistently appear in the seedling stage, although on most varieties they do not appear to a great extent until the plants have reached the jointing or boot stage. In fact, the spots usually develop most rapidly after the heading of barley.

The spotting usually, but not always, progresses from the lower to the upper leaves, the lower ones often being most severely injured. Spotting develops uniformly and simultaneously on nearly all the plants of the same variety that are in the same stage of development.

The spots may be scattered, as in the variety New South Wales Selection, or aggregated, as found in Kamet Mugi C. I. 2253. In fact, parts of leaves may be completely destroyed as a result of coalescence of numerous spots (Tatien C. I. 665), or they may become so weakened that they dry up or die from the attack of secondary organisms (Gorak C. I. 1086 and Cornutum C. I. 724). The spots produced on a given variety were similar in successive years. The characteristic symptoms, also, have been observed on many varieties when grown in the greenhouse. In most cases, however, the lesions were more distinct and larger in the field than in the greenhouse. In general, the type of spots produced is characteristic for the variety concerned, although a number of varieties may develop similar types.

EXPERIMENTS TO DETERMINE CAUSAL FACTORS

Failure to Isolate Pathogen

Numerous tests of many different sorts indicated that these leaf spots are, apparently, of nonparasitic origin and are either inherent or nutritional maladies. Many attempts were made to establish a parasitic origin but none were successful. Field and greenhouse tests indicate that healthy plants never become spotted by contact with badly spotted plants, even though their leaves and culms may be interlocking.

Attempts were made repeatedly to isolate causal organisms. Diseased leaves from many varieties grown in the field or greenhouse were plated out in the usual manner on nutrient agar. The results were always negative, except when leaves bearing old lesions were selected. From such material

different species of fungi and bacteria were isolated occasionally, but in every case they appeared to be of secondary importance. Likewise, leaves with newly formed leaf spots failed to develop fungi when placed in moist chambers.

Sections, mostly free-hand, but some microtome sections, were prepared from many different types of lesions obtained from a number of sources. Some of the cells in diseased lesions were collapsed, discolored, and dead, and in many of the adjoining cells there was a scarcity of chloroplasts. The progressive killing of the cells was observed microscopically but no causal organism was detected.[3]

In order to determine whether the different types of leaf diseases would be transmitted through the plant juices, extracts of leaves from 7 diseased varieties were injected with a hypodermic syringe into apparently healthy plants of 12 different varieties. The varieties of barley were sown late in May in 9 successive series. The leaves from the respective varieties were thoroughly macerated by mixing with quartz sand and grinding in a mortar. The desired quantity of sterile water was then added and the extract for inoculation was obtained by filtering. Distilled water and juice from a healthy variety were injected into 2 similar series used as checks. On June 22, the date of injection, the plants varied in development from the jointing to the early boot stage.

The nonparasitic diseases could not be induced by any of the above methods. In no case did any of the plants injected with juice from diseased leaves develop more lesions than noninoculated plants or the plants injected with sterile water or with juice from healthy leaves.

The environmental conditions certainly were favorable for the development of the disease, since on the control plots near-by, which, however, were planted earlier, the lesions increased very rapidly during this period. Furthermore, the conditions also were favorable for bacterial development. Some of the leaves of one variety, Yanehadaka C. I. 693, used in making juice extraction were infected with *Bacterium translucens* var. *undulosum* S. J. & R., and the 12 varieties injected with the juice developed the bacterial disease.

Effect of Cultural Conditions. The nonparasitic diseases seem to develop best during late spring and early summer, both in field and greenhouse. In 1931 and 1932, 125 varieties of barley were sown in replicated plots in April and again in late August. As already pointed out, in both years severe spotting occurred on many of the spring-sown varieties; but almost none on late-sown barleys. Similar results were obtained also in the greenhouse tests.

[3] The writer is indebted to T. W. Graham, a graduate student in Plant Pathology, for his efficient help in histological studies.

Most of the varieties of barley sown in the fall and winter in the greenhouse failed to develop typical leaf spots. When these varieties, however, were sown in the field or greenhouse in the spring they became severely spotted. The same response of the many varieties in 2 successive years suggested that the cause might possibly be a temperature relationship. But the greenhouse test with 10 varieties of barley grown at 2 distinctly different temperatures, ranging from about 16° to 22° C., and from about 20° to 30° C., respectively, failed to produce sharp differences in degree of spotting. Lesions produced at the high temperatures were frequently darker in color than those at low temperatures. Several similar tests with fewer varieties were made, but they also were negative.

In order to determine whether lack of moisture could induce the development of disease, 7 susceptible varieties of barley were sown in quadruplicate in porous pots. Two pots of each variety of barley were put in a large, flat pan in which the water was maintained at a level of 1–2 inches from the bottom. The other set of pots was watered just enough to keep the plants from wilting. No differences in degree of spotting between the 2 series was discernible at the end of the test.

Effect of Soil Type. Field observations indicate that the nonparasitic diseases develop on a wide range of soil type. In order to obtain definite information, greenhouse tests were carried out under controlled conditions.

In the spring of 1932 duplicate 4-inch pots of 25 different varieties of barley were sown in 4 types of soil; black loam, sandy soil, muck, and peat. These pots were all placed in a pit in the greenhouse and their positions were altered from time to time so as to give every plant as nearly as possible

TABLE 1.—*The comparative severity of nonparasitic lesions on 9 different varieties of barley when grown in the greenhouse on four different soil types. 1932*

Variety and C. I. No.	Type of soil and severity of disease[a]			
	Black loam	Sandy soil	Muck	Peat
Abyssinian C. I. 1216	H–L[b]	H	H	H
Algerian C. I. 1179	0	0	—	0
Black Hull-less C. I. 666	L	L	L	L
H. d. decorticatum C. I. 2230	H–	H	H	H
Lion C. I. 923	H–	H	L	L
New South Wales, Selection	L+	H	H	H
Peatland, Minn. 452	0	M	L–	0
Svanhals, Selection	T	M	L–	0
Trebi C. I. 936	M	L	H	—

[a] Notes were taken at boot or heading stage.

[b] H = heavy spotting; M = moderate; L = light; T = trace.

similar environment. The 9 varieties listed in table 1 are representatives of the 25 varieties on the different kinds of soil.

Twenty-four out of 25 varieties developed necrotic lesions somewhat similar to those observed in the field. There was considerable variation in the severity of the disease on certain varieties of barley on different types of soil. In general, the disease appeared to be most severe on the sandy soil and least severe on peat. It should be pointed out, however, that 8 varieties produced equally severe spotting on all 4 soils, but 8 other varieties developed more spotting on sandy soil than on the other types of soil. Some varieties developed decidedly more spotting on muck than on peat. Others were severely damaged by leaf spots on all 4 soil types and 1 remained clean.

In another test, in 1932, duplicate pots of 3 varieties were grown for 2 months on the following types of soil: peat, Clyde silt loam, Merrimac loamy fine sand, Gloucester fine sandy loam, Fargo clay, and a very rich garden soil. Notes were taken when the plants were in the jointing stage. In this test there was very little difference in the response of the varieties on the different soil types. Trebi, however, became much more heavily spotted on the Merrimac loamy fine sand than on the other 5 types. Severe leaf spotting occurred on a selection of *H. d. decorticatum* grown on 6 different soil types, while Gorak grown on rich garden soil was practically free, but developed a trace of spotting on the other soil types. In a later test in the spring Gorak became severely spotted. Thus, it would seem that the prevalence of lesions on a variety is not always governed by the same set of factors. Undoubtedly, the spotting on certain varieties is an expression of genetic factors as in Abyssinian C. I. 1216 and Yanehadaka C. I. 693 (Fig. 3).

Effect of Salts. A number of workers have shown that the presence or absence of certain salts may prevent or induce nutritional diseases (4, 5, 6, 7, 8). Therefore, the writer, in a preliminary way, tried the effect of adding one or more salts of aluminum, ammonium, barium, calcium, copper, iron, lead, lithium, magnesium, manganese, potassium, silver, boron, and zinc to the soil. Of the 20 compounds used only one, sodium borate, produced distinct spotting, although some compounds burned and even killed the leaves or plants. The spots produced by sodium borate and boric acid were similar to some of the nonparasitic spots and bore some resemblance to true spot-blotch lesions. The production of distinct lesions by the addition of boron to the soil is of considerable interest, since Eaton (2) is of the opinion that boron increases susceptibility of barley to spot blotch, *Helminthosporium sativum*, but renders it resistant to powdery mildew, *Erysiphe graminis* DC.

In order to test still further the toxicity of 16 compounds, freshly cut

culms of Minsturdi and Glabron barley were placed in Erlenmeyer flasks containing 5 different concentrations of each compound. As in soil tests, the boron compounds were the only ones that produced definite necrotic spots.

In a number of other tests it was shown that a trace of boron, either as boric acid or borax, could cause spot-blotch-like lesions. In fact, water cultures containing 1 part of boron in 10,000,000 produced numerous

FIG. 5. Lion, C. I. 923 and Trebi, C. I. 936 barley with boron leaf spots produced by adding 0.21 gm. of boric acid to 2,000 gms. of soil.

necrotic lesions on the leaves of healthy barley in 3 or 4 days. Higher concentration of boron produces similar effects but in proportionately shorter time. The type of symptoms produced by boron varies somewhat with amounts applied, stage of host, variety concerned, and humidity and temperature involved. The boron spots usually appear first on the lower leaves and on the tips of young vigorous growing leaves, especially along the margin. The spots may become so numerous that the leaves are destroyed, or even the entire plant may be killed. (Fig. 5). The boron spots that resemble most nearly the lesion produced by *Helminthosporium sativum* or other species of that genus are those produced on the leaves that continue to grow after the application of the boron. In many respects, though not in color, boron injury resembles the injury frequently observed on many of our common varieties of barley throughout Minnesota, especially on soil where barley does not thrive.

In still another test 11 varieties of barley were grown in 7-inch pots, each containing approximately 2,000 grams of garden soil. To one series of duplicate pots was added 50 grams of lime just preceding the sowing of seed. In two other series boric acid and copper sulphate solution were added, respectively, at 4 different times during seedling and jointing stages. The total amount of copper sulphate or boric acid solution given each pot was only .21 gram. Notes were taken when plants were in the jointing or

TABLE 2.—*The effect of applying solutions*[a] *of lime, copper sulphate, or boric acid to the soil in 7-inch pots in which were grown 11 different varieties of barley*

Variety and number	Treatment per pot and degree of spotting[b]			
	Control	Lime 50 gms.	$CuSO_4$ .21 gms.	Boric acid .21 gms.
Algerian C. I. 1179	0	0	0	M[c]
Black Hull-less C. I. 878	T+	L−	L−	M
Gorak C. I. 1086	M	M	L+	H+
H. d. decorticatum C. I. 2230	H	H+	H	H
H. d. deficiens, Selection	L−	L−	L−	H
H. v. duplinigrum C. I. 2206	0	0	0	0
Lion C. I. 923	T	T	T	H
Minsturdi, Minn. 437	T+	T+	T+	H
New South Wales, Selection	L	L	L	H
Purple Nudum C. I. 2250	M	M	M	H+
Trebi, C. I. 936	T	T	T	H+

[a] Each pot contained about 2000 gms. of soil. The lime was applied at time of sowing, but the boric acid and copper sulphate solutions were applied at 4 different times during the growing period of the barley.

[b] The notes were recorded at jointing or boot stage.

[c] H = heavy spotting; M = moderate; L = light; T = trace.

TABLE 3.—*The effect of applying boric acid[a] to the soil on the development of boron spots and powdery mildew on barley in the field and greenhouse*

Amount of boric acid per square meter	Variety and degree of infection or spotting[b]											
	Glabron				Manchuria				Minsturdi			
	Boron spots		Powdery mildew		Boron spots		Powdery mildew		Boron spots		Powdery mildew	
	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
10 gms.	M	H	L+[c]	H	M+	H	L+[c]	H	M+	—	T−[c]	—
20 gms.	H	H+	T	H	H−	H+	T	H	H	—	0	—
Control	0	0	M+	H+	0	0	M+	H+	0	—	T	—

[a] Boric acid was applied when the plants had developed 3 to 4 leaves.

[b] H = heavy infection or spotting; M = moderate; L = light; T = trace.

[c] The barley plants on the plots receiving boric acid were stunted, many of the leaves and even the plants were killed by boron injury; hence the density of foliage was much less than in the controls. Plants used in the greenhouse were "boron-diseased" plants transplanted from the field.

boot stage. The results are presented in table 2. As in previous tests, boric acid greatly stimulated production of necrotic lesions. All varieties tested were susceptible to boron injury. Lime and copper sulphate did not decrease or increase the amount of physiologic spotting over the controls.

RELATION OF BORON SPOTS TO HELMINTHOSPORIUM AND POWDERY MILDEW

A number of different tests were made to determine the effect of boron injury on the development of spot blotch and powdery mildew. The results are summarized in tables 3 and 4.

TABLE 4.—*The effect of applying solutions of sodium borate to the soil on the development of powdery mildew (Erysiphe graminis) and spot blotch (Helminthosporium sativum) when the plants were artificially inoculated in the greenhouse*

Variety	Stage of host development	Amount of sodium borate per pot	Degree of infection	
			Powdery mildew	Spot blotch
Black Hull-less, Selection	Boot	.1 gm.	M[a]	H
" "	Boot	none	L+	M
Lion, C. I. 923	Jointing	.1 gm.	M+	H
"	"	none	H	H
Minsturdi, Minn. 437	"	.1 gm.	T−	M−
"	"	none	L+	L+
"	Headed	.2 gm.	T−	M−
"	"	none	L+	M+

[a] H = heavy infection; M = moderate; L = light; T = trace.

The presence of boron in sufficient quantities to cause considerable spotting did not seem to increase susceptibility to spot blotch nor to render the plants immune from or even resistant to the attack of powdery mildew. In these studies, when barley plants with considerable boron injury were inoculated with mildew and placed in a favorable environment, heavy infection always developed on the healthy green portions of the leaves (Fig. 6).

An attempt was made to determine the relative tolerance of *Helminthosporium sativum* and *Erysiphe graminis* spores to boron. *H. sativum* spores germinated normally in solutions containing 1 part of boric acid or borax in 500 parts of water plus host tissue. Although a fairly high percentage of *E. graminis* spores began to germinate in this concentration, most of their germ tubes did not develop normally. At lower concentrations both species germinated normally. Thus it would appear that *H. sativum* spores have a greater tolerance to boron than mildew. Conidia of both species germinated normally in distilled water to which bits of "boron-diseased" tissue of barley were added. Even if one assumes that there is practically no boron present in the necrotic spots, powdery mildew could

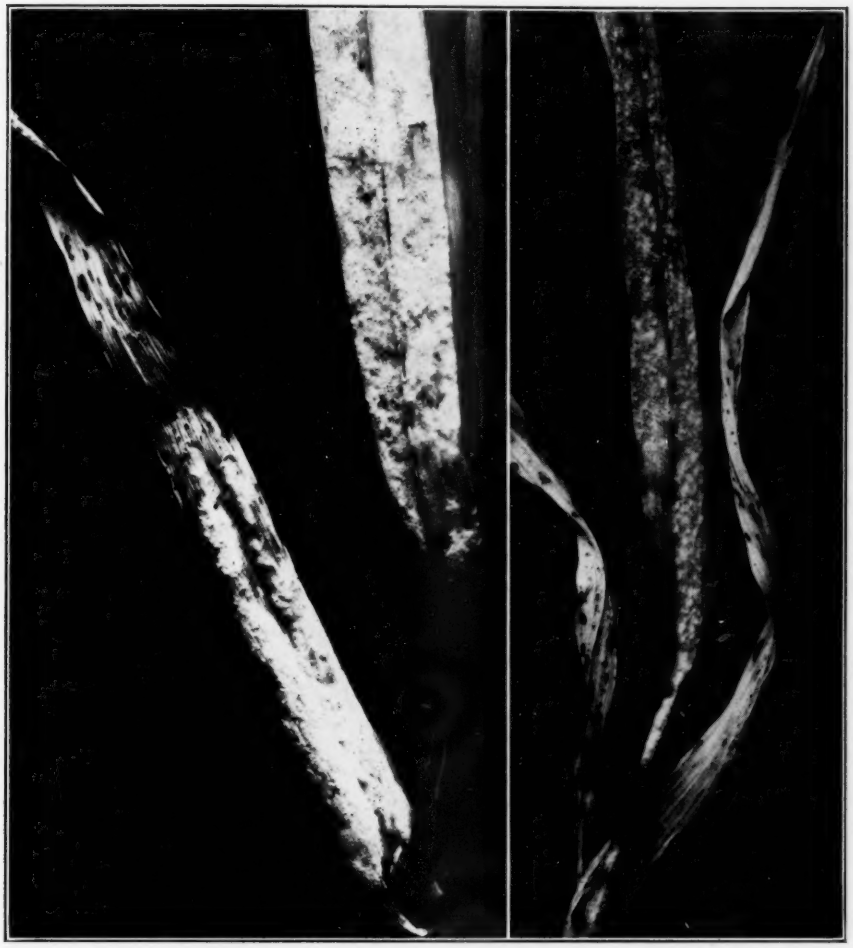

FIG. 6. Glabron Minn. 445 barley with considerable boron injury and heavy infection of powdery mildew. Note perithecial formation by the mildew.

not develop normally on the lesions, since it is an obligate parasite. On the other hand, *H. sativum* is a facultative parasite and grows very well on dead tissue. It is possible and also very likely that boron spots may serve as additional avenues of entrance for *Helminthosporium* spp.; but evidence obtained indicates that normal leaf tissues of boron-diseased plants are no more susceptible than those of uninjured plants.

CONTROL

Obviously, the leaf spots, if of nonparasitic origin, can not be controlled by seed treatment. One lot of seed from 4 varieties of barley was treated

with Ceresan, an organic mercury dust, at the rate of 3 oz. per bushel and another lot of the same varieties was treated by the Jensen modified hot-water method. Plants grown in the greenhouse from the seed developed as severe leaf spotting as those from nontreated seed.

It has already been pointed out that lime, copper, magnesium, and other compounds, when added to soil, failed to control the disease. It has been shown that certain nutritional diseases can be controlled by direct application of certain chemicals to the foliage (1). The writer also attempted to control the nonparasitic disease of barley by dusting and spraying. During the summer of 1932, 16 varieties of barley, 12 of which usually develop considerable leaf spot and 4 of which usually remain free from such disorders, were sown in 4 adjoining plots. One plot was sprayed with iron chloride 1:1000, and one with manganese sulphate 1:1000 on 4 different occasions about 7 to 10 days apart; the 3rd plot received several heavy applications of Kolo sulphur dust, and the 4th remained untreated. In no case did the treatment control, reduce, or intensify the disease. For example, the varieties Gorak C. I. 1086 and *Hordeum d. decorticatum* C. I. 2230 became heavily infected on all 4 plots, while 2 varieties, *H. v. duplinigrum* C. I. 2206 and Algerian C. I. 1179 remained essentially free on the 4 plots. The results were equally consistent for the 16 varieties tested.

The data in the first 3 columns in table 2 indicate that there are striking differences in varietal behavior to the physiological disease. Thus Algerian and *H. v. duplinigrum* remained free from spots, while Gorak and other varieties developed considerable infection.

DISCUSSION

It is evident that there are many nonparasitic leaf spots of barley. The symptoms are manifold and variable, depending on the cause of the spots and the environmental conditions under which they develop. The symptoms often are very similar to those caused by *Helminthosporium sativum* and other pathogens and can easily be confused with them. It has been shown definitely, however, that many of the spots are not caused by pathogens but by unfavorable soil conditions and inherent characters. In attempts to discover the soil factors responsible for the development of nonparasitic spots, boron was found to cause the development of spots quite similar to those caused by *H. sativum* and often indistinguishable from them. It seems very likely, therefore, that there has been confusion in the past with respect to the nature of many of the spots on barley. It is possible, also, that studies on varietal resistance have yielded questionable results because of confusion with respect to the cause of the spots. For example, the writer found that boron did not increase the susceptibility of

barley to *H. sativum* nor decrease its susceptibility to powdery mildew, as had previously been reported. This leads to the opinion that what has been considered spot blotch in the past was, in some instances, not spot blotch. It is apparent that the nature of parasitic and nonparasitic spots should be determined, for each variety used, in genetic studies and in tests of varietal resistance. It is possible that in many cases the existence of a large amount of nonparasitic spotting may serve as an indicator of the suitability of the soil for barley culture.

SUMMARY

In Minnesota, many different types of nonparasitic leaf spots are common on barley. The size, shape, color, and number and distribution of spots are variable. Many of the lesions are characteristic for a particular variety. Some of the lesions are similar to those produced by certain species of Helminthosporium and other fungi.

No evidence could be obtained that these spots were caused by a pathogen. No causal organisms were isolated. All evidence indicates that certain types of lesions are inherited, while others are the result of possible malnutrition or the presence of some toxic principle in the soil. Attempts to produce leaf spots with 20 different compounds were unsuccessful except with compounds containing boron.

It was demonstrated that boron, supplied either as boric acid or sodium borate, caused lesions that resembled spot blotch caused by *Helminthosporium sativum*.

Leaves of barley injured by boron were not rendered more susceptible to *Helminthosporium sativum* nor more resistant to *Erysiphe graminis* than nontreated plants. Less powdery mildew usually develops on boron-injured plants, chiefly because of the decreased area of healthy leaf tissue and because boron often causes premature killing of leaves and, consequently, less dense foliage.

The nonparasitic leaf spots could not be controlled by seed or soil treatment. Spraying with manganese sulphate and iron chloride and dusting with Kolo dust also were ineffective control measures.

Observations were made on severity of spotting on more than 125 varieties of barley. There were striking differences in their behavior. Some varieties always became severely spotted, others remained essentially free, while still others were intermediate in their behavior.

In some varieties the spots appear in the seedling stages, in others not until the boot or heading stage is reached.

Observations and tests in field and greenhouse over a period of years indicate that varietal behavior is relatively stable.

DIVISION OF PLANT PATHOLOGY
UNIVERSITY FARM
ST. PAUL, MINN.

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ISOLATION AND STUDY OF SOME YELLOW STRAINS OF CUCUMBER MOSAIC

W. C. Price

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INTRODUCTION

McKinney (7, 8) observed that tobacco plants infected with tobacco-mosaic virus frequently develop bright yellow spots in addition to the usual mottling symptoms. By cutting out the yellow spots and inoculating from them to healthy plants, he was able to isolate a virus that produced yellow-mosaic symptoms in tobacco. He suggested the possibility that yellow strains might arise from tobacco-mosaic virus by a process of mutation, using the term in its broadest sense. Jensen (4) also reported the occurrence of bright yellow spots in plants infected with tobacco-mosaic virus. He showed that not only one but a number of different yellow-mosaic viruses can be isolated from such spots. Jensen has shown further that these spots occur in plants infected with tobacco-mosaic virus that was separated from other viruses by successive passages through local lesions in *Nicotiana glutinosa* L.

Tobacco (*Nicotiana tabacum* L. var. Turkish) plants, infected with cucumber-mosaic virus, frequently develop bright yellow spots similar to those described for tobacco mosaic by McKinney and Jensen. From such spots, it has been possible to isolate several different viruses that produce yellow mosaic or necrotic symptoms in tobacco. Evidence has been obtained to show that these viruses are not the result of accidental infection but arise in infected plants by mutation or a similar process. The purpose of the present paper is to describe the isolation of and symptoms produced by these viruses and to present the evidence obtained in regard to their origin.

MATERIALS AND METHODS

Except where otherwise mentioned, a cucumber-mosaic virus obtained from Dr. R. H. Porter was used. The virus was designated by him (9) as "white pickle mosaic virus" or "cucumber virus 1." It is referred to in this paper as Porter's cucumber-mosaic virus. Several experiments were conducted with a strain of virus obtained from Dr. E. M. Johnson and classified by him (5) as "cucumber mosaic type 1 virus." When this virus is mentioned it will be referred to as Johnson's cucumber-mosaic virus. The two viruses are similar, but comparative inoculations to Turkish tobacco and other hosts have shown them to be different from each other.

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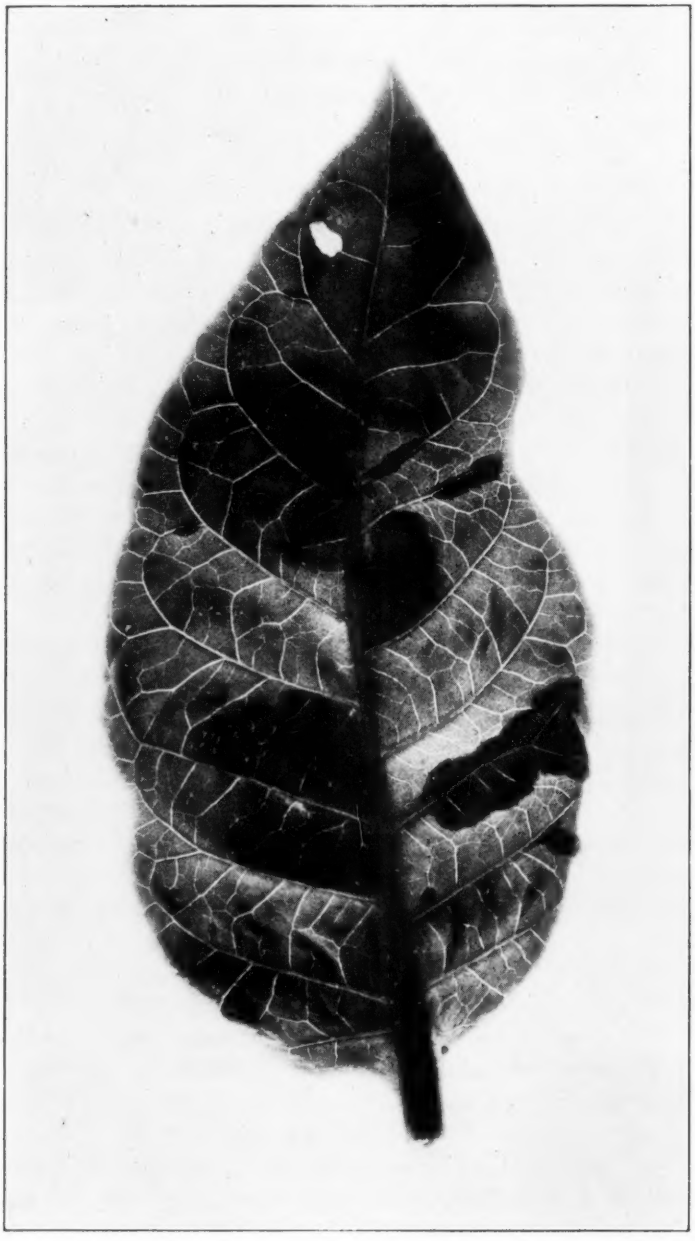

FIG. 1. A bright yellow spot in a leaf of a Turkish tobacco plant infected with Porter's cucumber-mosaic virus.

The symptoms produced by them in several hosts are described in a later section of the paper.

Reasonable care was taken to avoid accidental infection. Plants were grown in 4-inch pots in a greenhouse that was fumigated frequently to control insects. Inoculations were made by means of the rubbing method, except in a few instances in which the pin-puncture method was employed. Instruments used in making inoculations were sterilized by autoclaving or by boiling for a few minutes.

ISOLATION OF VIRUSES FROM YELLOW SPOTS

Bright yellow spots have been observed frequently in tobacco plants having cucumber-mosaic disease. They vary in size, shape, and intensity of the yellow color. They may develop within a few days after the appearance of the systemic disease, or they may not develop until plants have been infected for a month or more. Figure 1 shows a bright yellow spot that occurred in a tobacco plant infected with Porter's cucumber-mosaic virus. The conditions favoring frequent development of such spots have not been determined. The spots occur more frequently in plants making rapid growth than in slow-growing plants.

Inoculations were made from a number of bright yellow spots in order to determine whether viruses differing from Porter's cucumber mosaic could be isolated from them. Discs of tissue, each containing a single spot, were cut out with a sterile cork borer, macerated on a glass slide with a glass spatula, and rubbed over leaves of healthy tobacco plants. The glass spatulas used for making inoculations were similar to those described by Samuel (11). In 3 or 4 days, yellow local lesions developed in the inoculated leaves. Pin-puncture inoculations were made from the local lesions to healthy tobacco seedlings in flats. Out of a total of 956 single pin-puncture inoculations, infection was obtained from 14. In all cases, the first appearance of infection was the development of a chlorotic or necrotic area around the pin puncture. The infection gradually spread from these areas to other parts of the leaf and eventually involved younger leaves of the seedlings. Transfers were made from infected seedlings to healthy tobacco plants in pots. Five of the viruses thus isolated were selected for further study in order to determine the ways in which they differ from each other. A comparison was made of the symptoms produced by them in *Cucumis sativus* L. var. Davis Perfect, *Lycopersicon esculentum* Mill. var. Bonny Best, *Spinacia oleracea* L. var. Henderson's Long Season, *Nicotiana glutinosa* L., *N. langsdorffii* Schrank., and *N. tabacum* L. var. Turkish. Each of the viruses was tested by inoculation of 3 plants of each host. The results were confirmed by inoculation of one or more additional sets of 3 plants each. Controls were provided by rubbing an equal number of sim-

ilar plants with healthy juice. On the basis of the symptoms produced, all 5 of the viruses studied proved to be different from each other. They appear to be closely related to cucumber-mosaic virus and are considered to be strains of the latter.

SYMPTOMS PRODUCED BY VIRUSES ISOLATED FROM YELLOW SPOTS

In the following paragraphs, a description is given of the symptoms produced by 8 different strains of cucumber-mosaic virus. These strains include Porter's cucumber-mosaic virus, Johnson's cucumber-mosaic virus, 5 strains that were obtained by inoculation from yellow spots produced by Porter's cucumber-mosaic virus, and an additional strain of yellow mosaic (strain 6) that was isolated by inoculation from a plant infected with strain 5. All 8 strains have been passed in serial transfers through tobacco plants. With the exception of strain 5, they have produced symptoms that are constant.

Porter's Cucumber-mosaic Virus. The virus has been studied for a period of more than a year. It produces yellow primary lesions in *Nicotiana langsdorffii* and spinach, and, under certain conditions, inconspicuous yellow primary lesions in Turkish tobacco. The clearing-of-veins symptom is produced in cucumber, tomato, *N. glutinosa*, *N. langsdorffii*, and tobacco. The virus produces a coarse, green and light green mottling in all 5 of these hosts. The mottling in tobacco is illustrated in figure 2, A. Affected plants also show leaf distortion, blistering, crinkling, and stunting. In *N. glutinosa*, *N. langsdorffi*, and tobacco, the distortion and stunting are severe. These symptoms are usually less severe in cucumber and tomato. *N. glutinosa* and tobacco plants frequently show temporary recovery from the effects of the disease and develop several healthy-appearing leaves. In such instances, the next group of leaves to develop shows the typical mottling symptoms.

Johnson's Cucumber-mosaic Virus. This virus is characterized by the production of symptoms, similar, in general, to those resulting from infection with Porter's cucumber-mosaic virus. However, the two viruses may be readily differentiated by their reactions in certain hosts. In *Nicotiana glutinosa*, *N. langsdorffii*, spinach, tomato, and Turkish tobacco, the symptoms of the virus of Johnson's cucumber mosaic are milder than those of Porter's cucumber mosaic. In all 5 hosts, the former virus causes considerably less stunting and distortion. Tobacco plants that have been infected for periods of two weeks or more sometimes develop in their lower leaves a faint grayish necrosis that tends to follow small veins. This necrosis and the mottling that accompanies it are illustrated in figure 2, B.

Strain 1. This virus produces symptoms similar to those of the two viruses described above but that differ from them in certain characteristic respects. In all the hosts tested, with the possible exception of spinach,

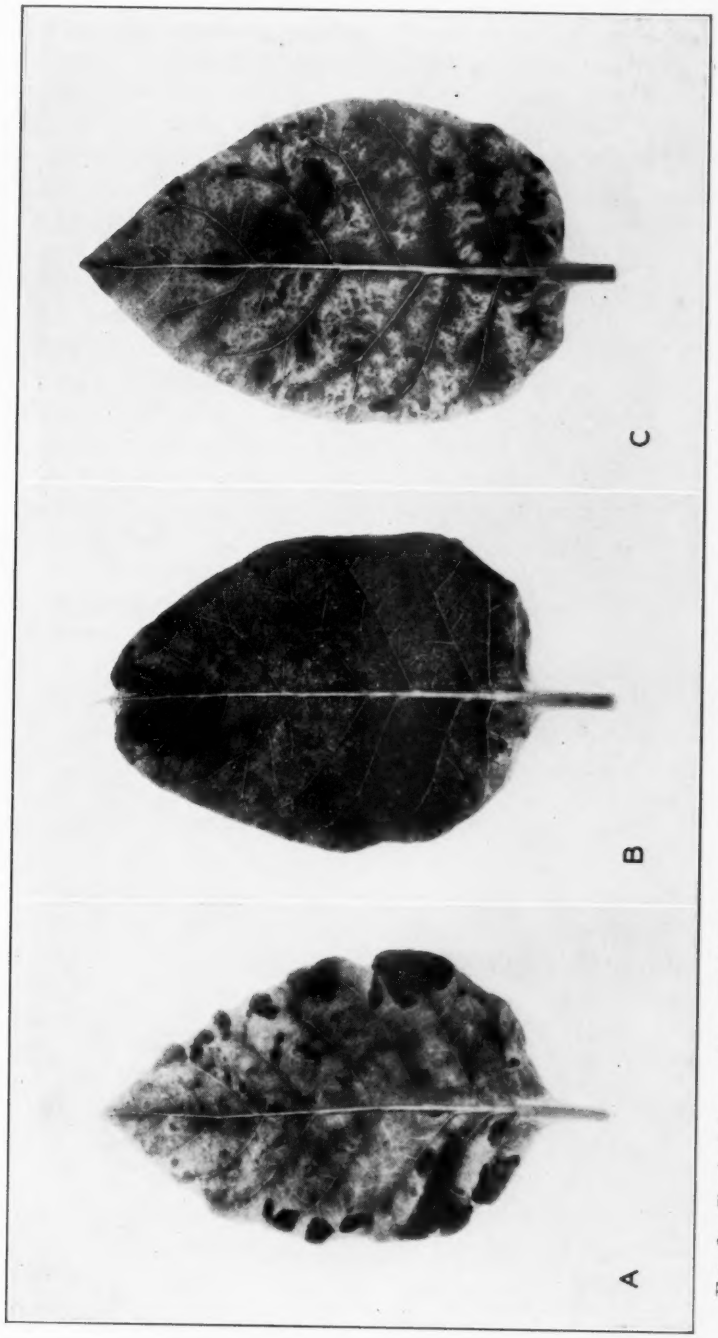

FIG. 2. Typical leaves from Turkish tobacco plants infected with cucumber-mosaic virus. A. Porter's cucumber mosaic. B. Johnson's cucumber mosaic. C. Strain 1.

it produces the least stunting and distortion of any of the cucumber-mosaic viruses so far studied. In *Nicotiana glutinosa, N. langsdorffii,* and Turkish tobacco, it produces grayish, necrotic primary lesions. In systemically diseased leaves of these hosts, it causes a yellow, blotch-like mottling frequently associated with yellow, zonate rings. In *N. glutinosa,* the yellowing tends to follow the larger veins. In tobacco, the yellowing appears in patches between the larger veins, as shown in figure 2, C. The yellowing in most of the hosts studied is more intense than that produced by either of the viruses described above but much less intense than that produced by some of the other strains that were isolated. In tomato, the virus causes only a mild mottling with almost no stunting or leaf distortion.

Strain 2. The symptoms produced by virus of strain 2 are similar to those of Porter's cucumber-mosaic virus but are much more brilliant. Bright yellow primary lesions are produced in spinach, tomato, *Nicotiana glutinosa, N. langsdorffii,* and Turkish tobacco. In the last 4 of these hosts, strain 2 causes a bright yellow or white, blotch-like, mottling and spotting. Figure 3, A, shows the type of mottling produced in tobacco. In cucumber, the mottling is more brilliant than that resulting from infection with the previously described viruses.

Strain 3. The symptoms produced by this virus in most of the hosts studied appear to be identical with those of strain 2. However, the two viruses may be differentiated by the symptoms they produce in *Nicotiana langsdorffii* and tomato. The virus of strain 3 moves a little more rapidly and produces less severe symptoms in *N. langsdorffii* and tomato than virus of strain 2. Tomato plants are not stunted quite so severely by strain 3 as they are by strain 2.

Strain 4. This virus, although apparently closely related to strains 2 and 3, produces less severe symptoms in some hosts. Usually the mottling is less brilliant and the stunting less severe in tomato, *Nicotiana glutinosa, N. langsdorffii,* and tobacco than that resulting from infection with either strain 2 or strain 3. Strain 4 produces a yellow or white mottling and spotting in all 4 of these host plants.

Strain 5. This virus produces symptoms strikingly different from those resulting from infection with the other strains studied. It causes necrotic primary lesions in tomato, *Nicotiana glutinosa, N. langsdorffii,* Turkish tobacco, and spinach, and yellow and necrotic primary lesions in cucumber. The first 4 of these hosts develop a systemic necrosis when infected. The necrosis is usually present as solid spots or zonate rings, but may occasionally follow veins and produce oak-leaf patterns. Figure 3, B, shows the symptoms produced by this strain in tobacco.

Strain 5 does not appear to be stable. In systemically diseased leaves of several of the hosts studied, it invariably gives rise to yellow-mosaic

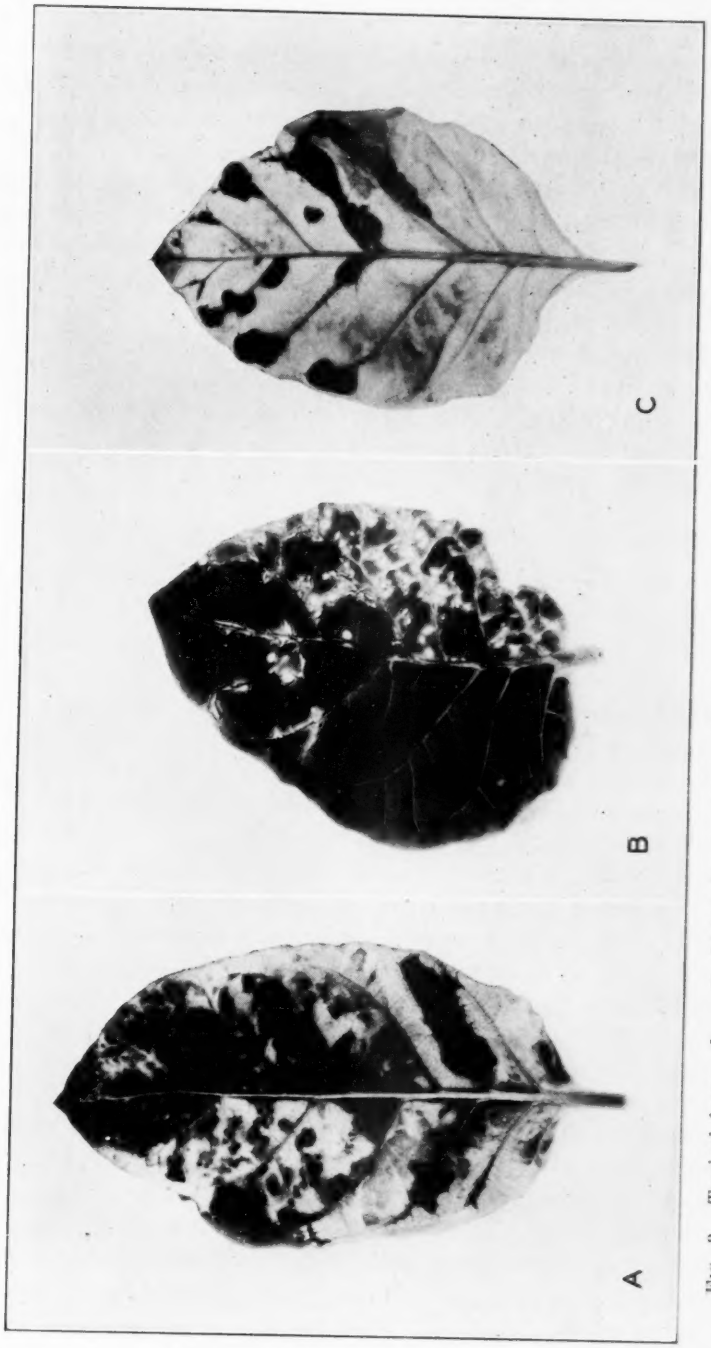

FIG. 3. Typical leaves from Turkish tobacco plants infected with cucumber-mosaic virus. A. Strain 2. B. Strain 5. C. Strain 6.

symptoms. By inoculating from leaves showing yellow-mosaic symptoms, a yellow-mosaic virus and a green-mosaic virus have been obtained free from the virus of strain 5. A description of one of these viruses (strain 6) is given below. The question of the origin of the yellow- and the green-mosaic viruses is considered in another section of the paper.

Strain 6. This virus was obtained by inoculation from the tip of a tobacco plant infected with strain 5. It produces the most brilliant symptoms of any of the viruses described in this paper. It causes yellow primary lesions in Turkish tobacco, cucumber, spinach, tomato, *Nicotiana glutinosa, and N. langsdorffi.* Systemic symptoms in all these plants consist of a mottling of brilliant yellow and dark green. Infected leaves of tobacco, *N. glutinosa*, and spinach occasionally become almost entirely yellow. More frequently, the yellowing is confined to certain portions of the leaves, the remaining portions being green. Figure 3, C, shows the symptoms of strain 6 in tobacco.

PROPERTIES OF THE VIRUSES OBTAINED FROM YELLOW SPOTS

The evidence reported in preceding paragraphs indicates that the viruses under discussion, although closely related, differ in the property (or properties) responsible for the expression of a symptom complex in the host plant. It is immediately of interest to know whether these viruses differ significantly in other properties. For this reason, tests were made to determine the thermal death points (for a 10-minute exposure) of the strains described above and their ability to withstand aging *in vitro.* The method used for heating virus samples was the same as that employed (10) in studies on the thermal death rate of tobacco-mosaic virus. For determining ability to withstand aging *in vitro,* the virus samples were kept in tightly stoppered 1 x 10 cm. test tubes in an oven held at 29° C. All tests were made with freshly extracted juice of mosaic-diseased tobacco plants. Infectivity of the virus samples was determined by inoculation of 10 young Turkish tobacco plants.

Results of tests on thermal death point are presented in table 1; those on aging *in vitro* are given in table 2. The tables show that there is little or no difference between the strains of cucumber-mosaic virus in thermal death point or ability to withstand aging *in vitro.* Since only a single test was made for each virus, the differences that were obtained may be the result of normal variation rather than the result of differences in reaction of individual viruses. It is possible, of course, that a more thorough study may reveal slight, but constant, differences. It is concluded that the viruses are similar in so far as their resistance to heating and to aging *in vitro* is concerned.

TABLE 1.—*Effect of exposure of different strains of cucumber-mosaic virus to various temperatures for 10 minutes*

Temperature in degrees C.	Porter's cucumber mosaic	Johnson's cucumber mosaic	Strain No. 1	Strain No. 2	Strain No. 3	Strain No. 4	Strain No. 5	Strain No. 6
60	3[a]	4	1	10	2	1	5	4
64	5	2	0	9	0	2	1	1
66	2	0	0	6	1	1	1	0
68	4	2	0	2	0	0	1	0
70	2	0	0	0	0	0	2	0
72	2	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0
Unheated controls	10	10	10	10	10	10	10	10
Healthy controls	0	0	0	0	0	0	0	0

[a] Figures represent the number of Turkish tobacco plants infected of 10 inoculated with the virus sample indicated.

TABLE 2.—*Effect of exposure of cucumber-mosaic virus at 29° C. for certain periods of time*

Time of exposure	Porter's cucumber mosaic	Johnson's cucumber mosaic	Strain No. 1	Strain No. 2	Strain No. 3	Strain No. 4	Strain No. 5	Strain No. 6
0 days	10[a]	10	10	10	10	10	10	10
1 day	9	10	3	10	0	8	4	3
2 days	5	10	2	0	4	5	3	0
3 "	4	10	0	1	0	0	0	0
4 "	0	1	0	2	0	0	0	0
5 "	0	0	0	0	0	0	0	0

[a] Figures represent the number of Turkish tobacco plants infected of 10 inoculated with the virus sample indicated. An equal number of controls, inoculated with healthy juice treated identically as diseased juice, remained healthy.

ORIGIN OF VIRUSES PRESENT IN YELLOW SPOTS

It having been shown that a number of different viruses can be isolated from yellow spots in tobacco plants infected with cucumber-mosaic virus, attention was directed to the question of the origin of such viruses. The frequent occurrence of yellow spots in mosaic-diseased plants, together with the failure to observe such spots in any of the healthy controls, proves that the viruses present in the yellow spots are not ordinarily the result of acci-

dental infection. Experiments were undertaken to determine whether such viruses were present in the original inoculum or had some other origin. An attempt was made to secure pure lines of cucumber-mosaic virus for use in these experiments. While there are several different methods for separation of closely related viruses, the primary-lesion method appears to be the most satisfactory for this purpose. This method was used in the present investigation.

In preliminary tests, it was found that leaves of several varieties of cowpea, *Vigna sinensis* Endl., would develop necrotic primary lesions when rubbed with cucumber-mosaic virus. Table 3 shows the results of these tests. The lesions appear within 2 days after inoculation and consist of circular, dark red, necrotic spots. They vary in size but usually are between 1 and 2 millimeters in diameter. Figure 4 shows the type of lesion that is produced in Black Eye cowpeas. The virus of cucumber mosaic is recoverable by subinoculation from the local lesions it produces in cowpeas.

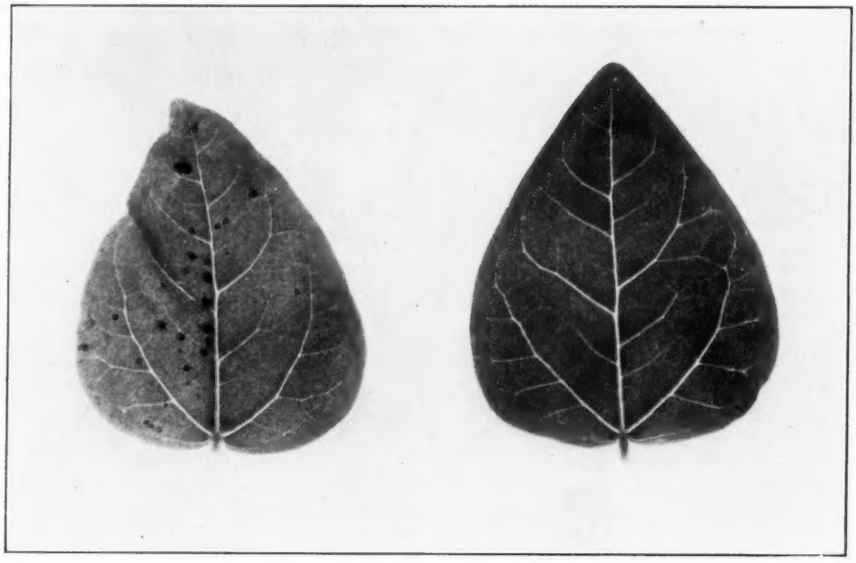

FIG. 4. Necrotic primary lesions produced in Black Eye cowpea by inoculation with Porter's cucumber-mosaic virus. A healthy leaf is shown for comparison.

For the purpose of securing a pure line of virus, Porter's cucumber-mosaic cirus was passed through successive transfers in primary lesions. Cowpea (var. Black Eye) plants were used in the series of transfers because the local lesions produced in this host, being necrotic, do not allow extensive spread of virus. After 10 successive transfers had been made

TABLE 3.—*Effect of inoculating varieties of cowpeas with cucumber-mosaic virus*

Variety	No. plants inoculated with juice of diseased plants	No. plants developing lesions	Total lesions	No. plants inoculated with juice from healthy plants (Controls)[a]
Black	72	46	965	67
Black Eye	63	40	184	21
Brabham	23	2	5	5
Clay	140	62	398	104
Early Buff	24	4	5	8
Groit	22	1	1	4
Iron	120	19	31	104
New Era	108	41	74	95

[a] Lesions did not develop in any of the controls inoculated by rubbing with juice of healthy plants.

and a pure line of virus was believed to have been established, the disease was transferred to tobacco plants for the purpose of determining whether or not yellow spots would be produced. Local lesions obtained on the 10th transfer were cut out with a sterile cork borer and used to inoculate healthy tobacco plants. Thirty-six tobacco plants were inoculated, a single lesion serving as inoculum for each plant. An equal number of controls were provided by rubbing leaves of tobacco plants with juice extracted from healthy-appearing tissues of the same cowpea leaves. Of the 36 plants inoculated with virus from local lesions, 25 became infected and developed symptoms typical of Porter's cucumber mosaic. A careful examination was made of the plants 3 weeks after inoculation. It was found that bright yellow spots had developed in 17 of them. Five of the plants showed 1 spot each, nine showed 2 spots each, two showed 3 spots, and one showed 4 spots. Eight of the infected plants had not developed yellow spots at the time the observation was made. All the controls were healthy and free from yellow spots. To determine whether or not yellow-mosaic viruses were present in the yellow spots, inoculations were made from 10 of the spots to healthy tobacco plants. All 10 of the inoculated plants developed yellow-mosaic and green-mosaic symptoms. It is concluded that yellow-mosaic viruses occur in tobacco plants infected with a green-mosaic virus previously passed through 10 successive transfers in primary lesions.

The experiment was repeated with Johnson's cucumber-mosaic virus after it had been subjected to 10 successive passages through cowpea local lesions. A number of the plants infected with this cucumber mosaic showed bright yellow spots. None of the controls showed yellow spots.

It is believed that a pure line was obtained by passing cucumber-mosaic virus through 10 successive transfers in primary lesions. It might be argued, however, that several strains of the virus were carried simultaneously in the same local lesions. In order to meet this objection, an attempt was made to separate two closely related viruses by passing them through local lesions in cowpea. Subinoculations were made to tobacco plants from single necrotic lesions produced in cowpeas by inoculation with a mixture of equal parts of a green (Porter's cucumber-mosaic virus) and a yellow (strain 2) cucumber-mosaic virus. Infection was obtained in 39 of the tobacco plants; 16 showed symptoms of green mosaic, 22 showed symptoms of yellow mosaic, and 1 developed symptoms of a mixture of green and yellow mosaics. From the tobacco plants that developed symptoms characteristic of infection with the green mosaic, only the green-mosaic virus was recoverable by inoculation to cowpea and subinoculation from single necrotic lesions. In the same way, only the yellow-mosaic virus was recoverable from the tobacco plants that developed symptoms characteristic of infection with yellow mosaic. From a single plant showing symptoms of a mixed infection, both viruses were recoverable by subinoculation. The results indicate that, except in a small percentage of the cases tested, the green-mosaic and the yellow-mosaic viruses were separated completely by passing them through necrotic local lesions in cowpea. It is realized that these results are not conclusive. It may be that the yellow-mosaic virus cannot be separated completely from the green. Nevertheless, the experiments add more weight to the conclusion that the strains of virus isolated from plants infected with a green-mosaic virus passed through 10 successive transfers in cowpea, were not present in the original inoculum but must have had some other origin. It is believed that yellow-mosaic viruses arise in plants infected with a green cucumber-mosaic virus.

ORIGIN OF VIRUSES ISOLATED FROM STRAIN 5

The virus of strain 5 produces necrotic lesions in Turkish tobacco. It is believed that the virus may be separated completely from other viruses with which it is mixed by successive inoculations from such necrotic lesions. When tobacco plants were inoculated with virus of strain 5, freed from other viruses by inoculations from a series of necrotic lesions, they invariably developed symptoms of yellow mosaic in addition to the usual necrotic symptoms. From such plants, a yellow-mosaic virus and a green-

mosaic virus were isolated. Evidence has been obtained to show that these viruses were not present in a mixture with strain 5, but that they arose in the plants infected with this strain. In a set of 83 tobacco plants infected with virus of strain 5, every plant developed local and systemic necrotic symptoms. All of the infected plants also developed yellow-mosaic symptoms eventually. Twenty-four showed yellow-mosaic symptoms on the 11th day after inoculation, 28 showed yellow-mosaic symptoms on the 14th day, 6 showed yellow-mosaic symptoms on the 16th day, 6 showed yellow-mosaic symptoms on the 22nd day, while 19 did not show yellow-mosaic symptoms until a month or more after inoculation. It is believed that if the yellow-mosaic viruses had been present in the original inoculum, all the infected plants would have shown yellow-mosaic symptoms at about the same time. Evidence for this belief was obtained in the following experiment. Ten tobacco plants were inoculated with virus of strain 5, 10 were inoculated with a sample of the same virus to which had been added virus of strain 6 in the proportion of 1 part of strain 6 to 9 parts of strain 5, while 10 similar plants were rubbed with water and held as controls. All of the 20 plants that were inoculated with strain 5 developed necrotic local lesions. The 10 plants that were inoculated with the mixture developed both yellow and necrotic local lesions. All 10 plants inoculated with the mixture developed systemic symptoms of yellow mosaic on the 7th day after inoculation. Those infected with strain 5 developed systemic symptoms on the 9th day after inoculation, but none of them showed symptoms of yellow mosaic until the 18th day. Most of them required an even longer time to show yellow-mosaic symptoms. It is concluded that both the yellow-mosaic and the green-mosaic viruses arose in plants infected with strain 5. Strain 5 appears to be more unstable than the other viruses isolated from bright yellow spots.

ALTERATION OF CUCUMBER-MOSAIC VIRUS IN PRIMARY LESIONS

In an experiment in which Johnson's cucumber-mosaic virus was passed from primary lesions in successive transfers, evidence was obtained that the virus may occasionally become altered in a primary lesion. Since this phenomenon is of considerable interest in connection with the origin of yellow-mosaic viruses and has not been reported previously, the evidence will be given here.

The virus of Johnson's cucumber mosaic was rubbed over leaves of several cowpea plants. One of the lesions produced was cut out and used to inoculate other cowpea plants. This inoculation may be spoken of as the first transfer. Controls were provided in the first and subsequent transfers by inoculation of an equal number of cowpea leaves with juice of a healthy cowpea plant. A pure line of virus was established by making

inoculations from a single, isolated lesion on the 2nd, 3rd, 4th, 5th, and 6th transfers. This line is represented by the letter G in table 4. On the 7th transfer, 3 related lines were established by inoculating from 3 single lesions of the 6th transfer. These are represented by G_1, G_2, G_3 in table 4. On the 9th transfer, an additional pure line was established by making inoculations from 2 necrotic lesions produced by line G_2 on the 8th transfer. Leaves inoculated with virus from one of these lesions developed both yellow and necrotic primary lesions. Subsequent transfers, referred to as Y_1 in table 4, were made only from the yellow lesions. On the 10th transfer, line G_3 developed both yellow and necrotic lesions. Subsequent transfers from this line, designated as Y_2, were likewise made only from the yellow lesions. Virus from the yellow primary lesions of lines Y_1 and Y_2 produced yellow lesions when inoculated into cowpea. The remaining 2 lines, G_1 and G_2, continued to yield only necrotic lesions in subsequent transfers. Cowpeas in which yellow lesions occurred developed symptoms that involved the young leaves and stem and eventually caused the death of affected plants. When transmitted to tobacco, on the 13th transfer, the virus present in the yellow lesions produced a yellow mosaic. Tobacco plants inoculated with virus from necrotic lesions of lines G_1 and G_2 developed green-mosaic symptoms.

TABLE 4.—*Serial transfers of Johnson's cucumber mosaic in cowpeas showing origin of strains Y_1 and Y_2*

Transfer No. 1 2 3 4 5 6 7 8 9 10 11 12 13	Symptoms produced in tobacco by inoculation from single lesions of the 13th transfer
$G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G_1 \rightarrow G_1 \rightarrow G_1 \rightarrow G_1 \rightarrow G_1 \rightarrow G_1 \rightarrow G_1$	Green mosaic
$G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G_2 \rightarrow G_2 \rightarrow G_2 \rightarrow G_2 \rightarrow G_2 \rightarrow G_2 \rightarrow G_2$	Green mosaic
$G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G_2 \rightarrow G_2 \overset{G_2^a}{\rightarrow} Y_1 \rightarrow Y_1 \rightarrow Y_1 \rightarrow Y_1 \rightarrow Y_1$	Yellow mosaic
$G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G \rightarrow G_3 \rightarrow G_3 \overset{G_3^a}{\rightarrow} Y_2 \rightarrow Y_2 \rightarrow Y_2 \rightarrow Y_2 \rightarrow Y_2$	Yellow mosaic

[a] Transfer in which yellow primary lesions first appeared. Subsequent transfers were made only from the yellow lesions in these two lines.

On the 13th transfer, the virus of line Y_1 and Y_2 was inoculated into additional sets of cowpea plants. Yellow primary lesions were produced. In many cases, but not all, a necrotic ring partially or completely en-

circled the lesion. When the disease became systemic, affected plants were not killed as in previous instances but developed a green and yellow mottling in the newly formed leaves. The disease is illustrated in figures 5 and 6. Virus from the mottled leaves has produced in all cases up to the present time yellow primary lesions, with or without a necrotic periphery, followed by typical mosaic mottling in systemically affected leaves. It is not yet known whether the virus was again altered by further passage through cowpea or whether the conditions of the experiment modified the course of the systemic infection.

The yellow-mosaic virus of lines Y_1 and Y_2 differs from all the other yellow-mosaic viruses previously referred to (strains 1, 2, 3, 4, 5, and 6), since none of the others produce yellow primary lesions nor become systemic in cowpea plants to which they are transferred. This strain of cucumber-mosaic virus produces a severe mosaic disease in cowpeas.

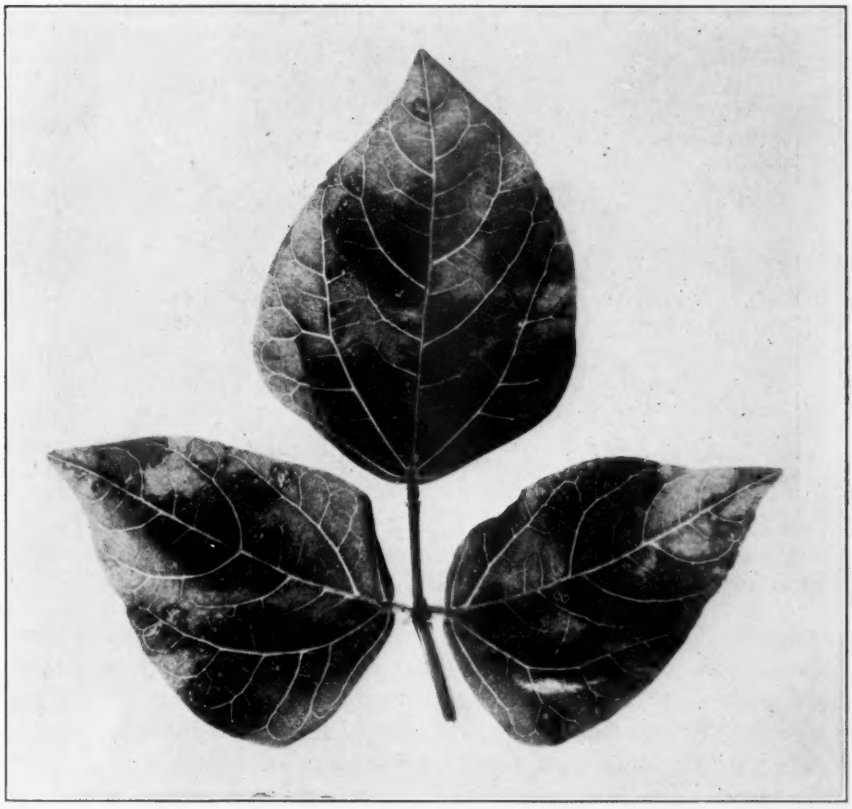

FIG. 5. Mottled leaf of Black Eye cowpea infected with cucumber-mosaic virus, strain Y_1.

DISCUSSION

The results obtained in this investigation have established the fact that several different but closely related yellow-mosaic viruses can be isolated from bright yellow spots produced in tobacco plants by inoculation with ordinary green cucumber-mosaic virus. It has been shown further that these viruses are not the result of accidental infection and that they may occur in plants infected with a green-mosaic virus that previously was passed through a series of primary necrotic lesions. While it can be argued that a mixture of several cucumber-mosaic viruses might be carried simultaneously through serial transfers in local lesions, evidence has been obtained to show that this is not the case. It was found that 2 closely related cucumber-mosaic viruses may be separated completely, so far as could be determined with the experimental methods available, by passing them

FIG. 6. Black Eye cowpea plants infected with cucumber-mosaic virus, strain Y_2, showing severe stunting, mottling, and distortion. Healthy controls on the right are presented for comparison.

through local lesions in cowpea. Similar results were obtained with tobacco-mosaic virus by Jensen (4) and by Kunkel (6). Jensen (4) presented evidence to show that 2 closely related tobacco-mosaic viruses could be separated by inoculation from single local lesions produced in *Nicotiana glutinosa* with a mixture of the 2 viruses. Kunkel (6) showed that from single necrotic lesions produced in *N. langsdorffii* by inoculation with mixtures of tobacco- and aucuba-mosaic viruses, one or the other of the viruses, but seldom both, is recoverable by subinoculations to *N. sylvestris*.

On first thought, it might seem that the sudden appearance of the yellow-mosaic viruses, Y_1 and Y_2, in cowpeas after 8 successive passages of a green-mosaic virus constitutes evidence that the primary lesion method is not suited for separation of closely related strains of virus. It is recognized as a possibility that these viruses might have been present as a mixture in the necrotic lesions with the green-mosaic virus and that separation was not accomplished until after 8 successive transfers had been made. On the other hand, it seems probable that if the yellow-mosaic virus had been present in the first passage it would have become systemic in the cowpea plants to which it was transferred. It appears, therefore, that the explanation which best fits the facts in the case is that only the green-mosaic virus was present in the first 7 transfers and that it suddenly became altered and gave rise to a yellow-mosaic virus. It is difficult to avoid the conclusion that yellow-mosaic viruses arise in plants infected with cucumber-mosaic virus. Such viruses arise in primary lesions, as well as in systemically diseased leaves.

The exact manner in which yellow-mosaic viruses are produced has not been determined. Assuming a chemical nature for the virus, it may be that new strains arise by a rearrangement or isomerization of a group or groups in the virus unit. A second possible explanation for the origin of yellow-mosaic viruses is that they are derived from a green-mosaic virus by a process similar, in general, to mutation in living organisms. It is left for future work to show whether this change in the virus results from a sudden alteration in a particular virus unit and subsequent multiplication of that unit, whether the virus in a certain cell of the plant host stimulates the production by that cell of another strain of virus, or whether there is some entirely different explanation.

One of the strains of cucumber-mosaic viruses isolated from yellow spots was found to be unstable. From a necrotic-type virus (strain 5), it has been possible to isolate a yellow-mosaic and a green-mosaic virus. Evidence was obtained to show that neither the yellow-mosaic nor the green-mosaic virus was present in the original inoculum, but that both viruses arose in infected plants. The other strains isolated from yellow spots may also prove to be unstable on further study.

Whatever the origin of yellow-mosaic viruses, they are readily obtained from Porter's cucumber-mosaic virus and presumably from other field types of cucumber mosaic. It is probable that the development of strains of cucumber mosaic is much more common than one would be led to suppose from the frequency with which bright yellow spots occur in infected plants. Strains that do not produce such a marked effect in the tissues of the leaf may also be present. It is possible that a plant infected with a single strain of virus may eventually come to contain several green

or masked strains, as well as one or more yellow strains. The green and masked strains would be difficult to detect unless they were separated from other strains present in the same plant.

Many different strains of cucumber-mosaic virus are known to occur in nature. Jagger (3) distinguished between the ordinary "white-pickle" mosaic and a second mosaic that mottled the leaves of cucumber but showed no effect on fruit. Bewley (1) observed 2 types of cucumber mosaic in Great Britain. Johnson (5) distinguished 3 types of cucumber mosaic that occur in tobacco in Kentucky. Two additional types of cucumber mosaic were observed recently in Kentucky (2). Porter (9) described a cucumber mosaic differing from the ordinary mosaic and characterized by the development of yellow blotches. McKinney (8) describes yellow mosaics occurring in the cucurbits *Momordica charantia* L. and *Kedrostis foetidissima* Cogn. in West Africa. The conclusions arrived at here in regard to the origin of yellow- and green-mosaic viruses offer an explanation for the occurrence of the many different strains of cucumber-mosaic virus present in nature. The same phenomena may explain the occurrence of so many different virus diseases of plants. It should be pointed out, however, that, so far as they have been tested, the strains of cucumber mosaic that were isolated from yellow spots differ only slightly in their properties and appear to be closely related viruses. It has not yet been shown that one plant virus may give rise to a second virus differing as markedly in its properties from the first virus as, for instance, cucumber-mosaic and tobacco-mosaic viruses differ from each other.

On the other hand, it has been shown that a strain of cucumber-mosaic virus, producing only necrotic primary lesions in cowpea, may give rise to another strain that becomes systemic and produces a severe mosaic disease in this host. Whether this strain of cucumber mosaic is identical with the cowpea mosaic described by Smith (12) is not known. The results reported here suggest that new strains of viruses capable of attacking entirely new host plants may arise naturally in the manner described.

SUMMARY

Tobacco plants infected with cucumber-mosaic virus frequently develop bright yellow spots from which different but closely related yellow-mosaic and necrotic-type viruses may be isolated. When the virus of cucumber mosaic is passed in serial transfers through the necrotic primary lesions that it produces in cowpea, *Vigna sinensis* Endl., and is subsequently inoculated to tobacco, it continues to produce bright yellow spots in addition to the usual mottling symptoms. Evidence is thus obtained that strains of cucumber-mosaic virus arise by mutation or a similar process in tobacco plants having the cucumber-mosaic disease. Evidence also was obtained to

show that strains of cucumber-mosaic virus may arise in primary lesions, as well as in systemically diseased leaves of affected plants. One of the strains of virus isolated from bright yellow spots was found to be unstable and to yield additional strains on subinoculation. With one exception, the strains of cucumber-mosaic virus studied produce necrotic primary lesions in cowpea but do not become systemic in this host. However, a strain obtained by serial passages through such necrotic lesions causes a typical mosaic disease in cowpea.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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INHERITANCE OF RESISTANCE TO BUNT AND LEAF RUST IN THE WHEAT CROSS, ORO × TENMARQ[1]

C. A. WISMER[2]

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INTRODUCTION

Bunt of wheat, caused by *Tilletia levis* Kühn, is one of the most serious diseases of wheat in Kansas, where, according to Melchers (14), it caused a loss of about 15,000,000 dollars in 1926. Since it is difficult to impress the wheat grower with the importance of seed treatment as a method of bunt control, the most satisfactory solution would seem to be the use of resistant varieties. The problem of producing such varieties has also presented the opportunity to study the inheritance of resistance to bunt.

This paper deals primarily with the manner of inheritance of resistance to *Tilletia levis,* in the wheat cross, Oro × Tenmarq. It also considers briefly resistance to leaf rust (*Puccinia triticina* Eriks.) in this cross.

REVIEW OF LITERATURE

The production of bunt-resistant varieties of wheat was one of the successes of William Farrer (9), the well-known pioneer Australian plant breeder. Since that time, many crosses have been made in the United States and other countries in attempts to secure bunt-resistant varieties of wheat embodying those characters necessary to make them popular with both the farmer and the miller. In recent years investigators have studied wheat hybrids to determine as nearly as possible the mode of inheritance of resistance to bunt.

Gaines (10) concludes from a study of the resistance to bunt in Turkey × Hybrid 128 and Turkey × Florence that most of the factors for resistance are recessive. He considers that the inheritance of resistance to bunt is due to multiple factors and that all varieties of wheat do not possess the same factors for resistance.

Gaines and Singleton (12) and Aamodt (1) reported transgressive segregation in a number of F_3 lines. Briggs (3, 4, 5, 6, 7), in researches involving one physiologic form of the bunt organism, concludes that the inheritance of resistance to bunt is governed largely by 1 dominant factor, as in Martin, White Odessa, Banner Berkeley, and others, or 2 such factors, as in Hussar. He believes there may be other factors for resistance in these

[1] Contribution No. 336 from the Dept. of Botany, Kansas State College of Agriculture and Applied Science.

[2] Formerly Graduate Assistant, Dept. of Botany, Kansas State College. The writer wishes to express his appreciation and gratitude to Dr. John H. Parker and Prof. L. E. Melchers, under whom these studies have been carried on, for supplying the materials used in this study and for the helpful suggestions and criticisms.

varieties, which would become evident if they were tested with other physiologic forms of the bunt organism (6). Bressman (2) found the deviation from the expected too great in the Hybrid 128 × Martin cross to explain the resistance of Martin to 3 collections of smut on a 1-factor basis. Churchward (8), working with Florence × Hard Federation, obtained evidence that seemed to show the presence of a 1-factor difference for resistance to bunt in which susceptibility was dominant. Aamodt (1) and Kilduff (13), from a study of inheritance of resistance to bunt, obtained evidence indicating the presence of several factors governing reaction to bunt.

METHODS AND MATERIALS

The cross, Oro C.I. 8220 × Tenmarq Sel. Ks. No. 2637, was made by John H. Parker of the Kansas Agricultural Experiment Station, the purpose being to combine the desirable characters of Tenmarq, namely, excellent grain quality, high yielding capacity, and earliness, with the bunt resistance and winter hardiness of Oro.

PARENTAL VARIETIES

Oro is a pure-line selection made by H. M. Woolman in 1921 at the Sherman County Branch Station, Moro, Oregon, from a variety labeled Turkey No. 889, received from the United States Department of Agriculture. Tests at the Moro Station showed this selection very resistant to bunt and agronomically promising.

Tenmarq is the product of a cross between P1066, a strain of Crimean hard red winter wheat similar to Kanred, and Marquis. The cross was made in 1917 in the botany rust nursery for the purpose of combining the stem-rust and leaf-rust resistance of Kanred with the earliness and superior milling quality of Marquis. The variety Tenmarq originated from hybrid plant No. 16A12–5815–2, later known as Sel. No. 215421. The row originating from this plant was selected by Parker because of its high yield, apparent superior grain quality, stiff straw, and freedom from leaf rust.

Tenmarq is susceptible to bunt, while Oro is resistant, as shown by several years' results.

TABLE 1.—*Comparison of Oro and Tenmarq in regard to reaction to bunt at Manhattan, Kansas*

Variety	Annual incidence of bunt					Average bunt incidence
	1929	1930	1931	1932	1933	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Tenmarq	46.0	26.1	23.3	57.0	33.5	37.2
Oro	1.1	1.8	0.1	5.0	0.4	1.7

Tenmarq has been rather widely tested at other experiment stations and has been grown in cooperative experiments on Kansas farms since 1927. In 1932 the Kansas Crop Improvement Association approved Tenmarq as a standard variety of hard red winter wheat for certification, and about 520 bushels of seed were distributed to farmers in August, 1932. About 6,000 bushels of certified seed of Tenmarq were produced in Kansas in 1933.

Hybrid Populations

Hybrid populations of Oro × Tenmarq used in these studies consisted of 2 F_2 populations, each from an F_1 plant, 16 F_3 lines, and 183 F_4 rows. Individual plant selections of F_2 and F_3 hybrid material were made for the F_3 and F_4 generations, respectively, on the basis of number and uniformity of tillers, stiffness of straw, weight of heads, and apparent vigor.

Each plant was numbered and threshed separately. Notes were taken on the threshed grain and further selection was made on the basis of plumpness, weight in grams per plant, corneous texture, and freedom from yellowberry.

The studies on the inheritance of resistance to bunt in 1932 were divided into 2 parts: (*a*) a detailed study of the reaction of F_2, F_3, and F_4 hybrid selections when inoculated with bunt designated as physiologic form 1, from Lincoln County, Kansas; and (*b*) determination of the relative resistance of F_4 selections when inoculated with a composite of 19 collections of bunt representative of Kansas. This composite includes 7 physiologic forms identified by Melchers,[3] but the larger number of collections have been grouped in the class designated as physiologic form 1.

For the studies in (*a*), the seed was thoroughly blackened with bunt spores of form 1, propagated on Kanred the previous season. The seed was space-sown approximately 3 inches apart in rod rows. Parental check rows were sown every 10th row. The entire nursery was sown the same day, so that soil moisture and temperature conditions were very similar for all of the selections.

The soil moisture was calculated from soil samples taken at a depth of approximately 2 inches, which was the normal depth at which the nursery was seeded. The moisture percentages varied from 15.4 per cent at one end of the series to 19.4 per cent at the other.

A continuous soil temperature record was obtained with a soil thermograph that recorded the temperature at a depth of about 2 inches. The soil temperature for the week following sowing varied from 42° to 70° F. with an average of 58.8° F. for this period. The lowest temperatures were recorded during the first few days after planting.

At harvest, the plants in each row were pulled and separated into 3 lots,

[3] Unpublished data.

as follows: (1) Those in which the plants were totally bunted; (2) those partially bunted; (3) those that were bunt-free.

Gaines (11), in dealing with partially smutted plants, used the formula, $ab + c = d$. In this formula a = the percentage of bunted heads on a partially bunted plant, b = the percentage of partially bunted plants in a row, c = the percentage of entirely bunted plants in a row, and d = the total percentage of the row that is bunted. This gives a satisfactory quantitative measure, which he believes important in studying resistance. Briggs (3), however, objects to this method because it does not indicate the nature of segregation.

In the experiments here reported all smutted plants, regardless of the degree of smutting, were recorded as totally diseased in calculating the bunt percentages used in the study of the inheritance of bunt resistance.

The experiments under (*b*) deal with 183 F_4 selections from individual F_3 plants inoculated with a composite of Kansas bunt forms. These selections were made at random as far as resistance or susceptibility to bunt was concerned. This series was seeded in 5-foot rows 1 foot apart. The soil moisture in this plot at time of planting was 17.7 per cent, while the soil temperature was about the same as that for the space-planted series.

Since no selections were to be made from this section of the nursery, 4 feet of each row in this series were cut during June and the bunt counts made as soon as all the heads were in bloom so that the diseased heads could easily be identified. Plants in 1 ft. of each row were left standing until a heavy leaf-rust infection had reached full development. Leaf-rust notes were taken by C. O. Johnston and the writer.

To provide material for the experiments in 1933, individual plant selections were made in the summer of 1932, just as in the previous season. The individual plants were threshed and grain notes taken. Individual plants in the most resistant lines within each of the 3 generations showing desirable grain characters were selected for sowing. In addition several plants in rows showing approximately 20, 40, 60, and 80 per cent infection in 1932 were selected from the F_3 and F_4 material for sowing. These lines were inoculated with a composite of Kansas bunt forms and space-planted in 8-ft. rows.

Because of the dry weather at harvest time, the plants could not be pulled, so the bunt percentages are based on total number of culms and bunted heads rather than on the percentage of bunted plants.

EXPERIMENTAL RESULTS

Bunt Reaction of Parental Varieties and Hybrids to Physiologic Form 1

One physiologic form of the bunt organism was used to study the manner of inheritance of resistance to bunt in hybrid populations of Oro × Ten-

marq. Parental check rows of Oro had an average of 7 per cent of bunt. Tenmarq showed an average of 43 per cent. The distribution for parents and hybrid progenies into 5 per cent classes for bunt infection is shown in table 2.

TABLE 2.—*Distribution of rows of Oro, Tenmarq, and their hybrid progenies into 5-per-cent classes for infection produced by physiologic form 1 of Tilletia levis*

Bunt	Parents		Hybrids		
	Oro	Tenmarq	F_2	F_3	F_4
Per cent					
0	1			2	66
0–4.9	2			4	17
5–9.9	5			1	11
10–14.9	3		1	4	8
15–19.9	1			2	13
20–24.9		1	1	1	18
25–29.9		2		1	5
30–34.9		3			5
35–39.9		1			4
40–44.9		5			5
45–49.9				1	7
50–54.9		1			2
55–59.9				1	6
60–64.9		1			6
65–69.9					6
70–74.9					1
75–79.9					2
Ave. per cent	7	43	16.8	15	18.2
Total No. rows	12[a]	14	2	17	182[a]

[a] Percentage bunt for 1 row was not recorded.

These hybrid populations previously had never been infected with bunt, but selection for desirable agronomic characters had been carried on in each generation.

Two F_2 populations from individual F_1 plants in 1931 had averages of 20 per cent and 13 per cent of bunt. These are intermediate between the 2 parents in bunt infection. It is of interest to note that these hybrid populations each contain only about 1 per cent of totally smutted plants. Of the parental rows grown immediately adjacent to these F_2 populations, the resistant parent, Oro, shows 0 per cent, while Tenmarq, the susceptible parent, shows 12.5 per cent of totally smutted plants.

The F_3 population consisted of 17 lines from individual F_2 plants grown in 1931. Two of these F_3 lines showed no bunt whatever, and 4 showed

infection ranging from 0 to 5 per cent. Two F_3 rows showed greater susceptibility than the average of the Tenmarq parent. The greater number of bunt-infection percentages are grouped about the mean of the Oro parent. The number of F_3 lines is far too small for genetic analysis.

The F_4 population is made up of 183 lines from individual plants selected from 23 F_3 lines grown in 1931. Sixty-six rows showed 0 per cent bunt, while an additional 17 rows showed bunt infection no greater than 0 to 5 per cent. Thirty-one rows showed a greater susceptibility than the average of the Tenmarq parent. The larger number of bunt-infection percentages are grouped near the mean percentage of bunt infection of Oro, the resistant parent.

The distribution of bunt infection percentages of F_4 progenies, grouped according to F_3 parental lines, is shown in figures 1, 2, and 3. The dots on the base line are used to indicate 0 per cent of bunt infection. The pedigree numbers show the number of F_4 progenies from each F_3 line designated by the 1931 row numbers. Within each F_3 family the F_4 progenies are arranged in order from that with the lowest to that with the highest average percentage of bunt. The average per cent of infection of the F_4 lines from each F_3 family is shown by a line designated as such in the figures.

Resistant F_4-progeny rows from 8 F_3 lines, which were evidently homozygous for resistance to bunt, are included in figure 1. The F_4 lines from the 8 F_3 families range from an average of 0 per cent of bunt to 1 per cent, with a maximum of 8.5 per cent for one F_4 progeny row.

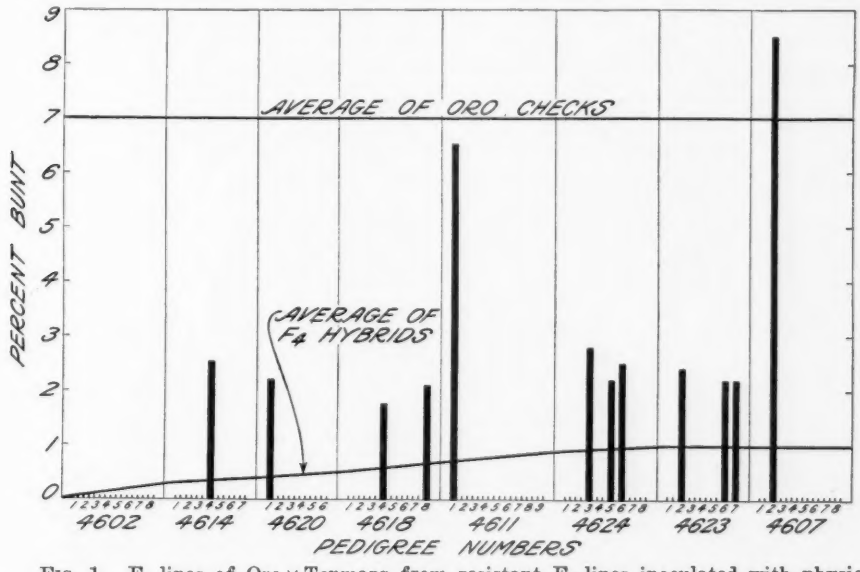

FIG. 1. F_4 lines of Oro × Tenmarq from resistant F_3 lines inoculated with physiologic form 1 of *Tilletia levis*.

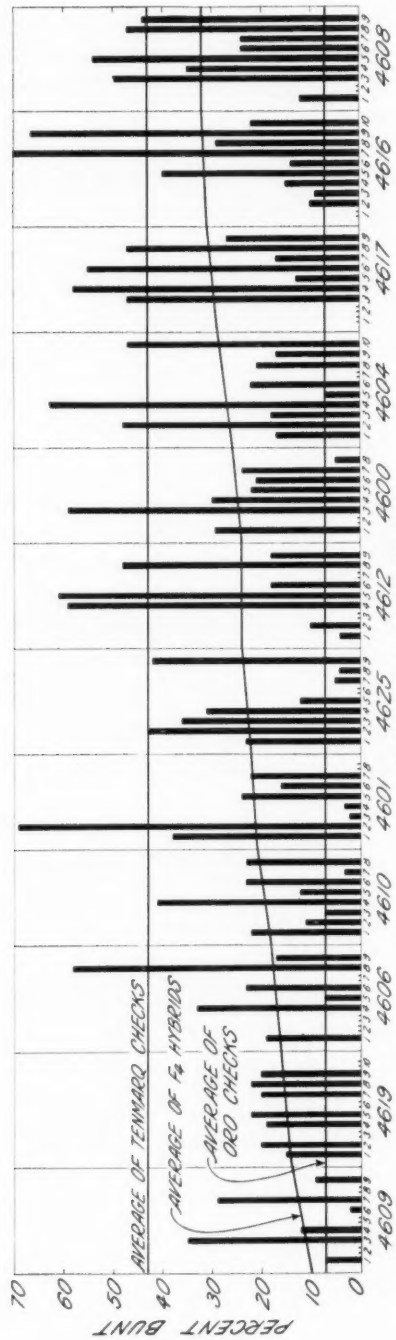

FIG. 2. F_4 lines of Oro × Tenmarq from F_3 lines heterozygous for bunt resistance, inoculated with physiologic form 1 of *Tilletia levis*.

Resistant and susceptible F_4 lines from 12 F_3 lines that were evidently heterozygous for bunt reaction are grouped in figure 2. The F_4 lines from F_3 lines included in this figure range from an average of 10 per cent to 32 per cent of bunt infection. Individual F_4 lines within the F_3 groups range, for the most part, from 0 bunt infection to more than the average for the susceptible Tenmarq parent. There is unmistakable evidence of clear-cut segregation for bunt resistance among the F_4 lines from each of the F_3 lines here represented.

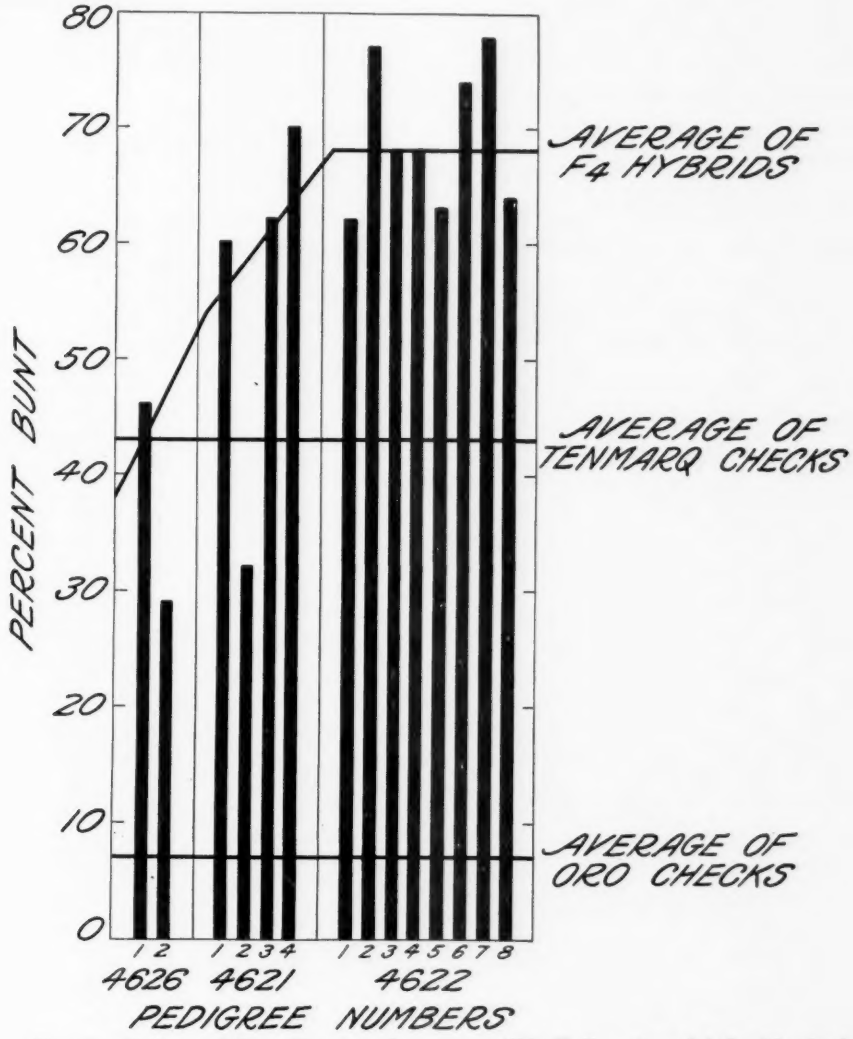

FIG. 3. F_4 lines of Oro × Tenmarq from susceptible F_3 lines inoculated with physiologic form 1 of *Tilletia levis*.

Susceptible F_4 lines from susceptible F_3 lines are represented in figure 3. These F_3 lines evidently were homozygous for susceptibility, although the number of F_4 lines representing 2 of the F_3 lines is rather small. The F_4 lines representing F_3 groups show an average bunt infection of 38, 55, and 69 per cent, respectively, for form 1. These lines show a higher bunt infection when inoculated with the composite of bunt collections.

As shown in these 3 figures representing F_4 lines, the parental F_3 lines may be classed as homozygous-resistant, heterozygous, and homozygous-susceptible, in the proportion of 8 : 12 : 3, respectively. The number of F_3 lines represented is too small for genetic analysis, though high susceptibility evidently is recessive in this cross.

Reaction of Parental Varieties and Hybrids to a Composite of Kansas Bunt Collections, 1932

When inoculated with a composite of Kansas bunt forms, Oro has an average of 5 per cent and Tenmarq of 57 per cent of bunt. The distribu-

TABLE 3.—*Distribution of bunt infection percentages of Oro, Tenmarq, and F_4 lines into 5-per-cent classes when inoculated with a composite of Kansas bunt forms*

Bunt	Oro	Tenmarq	F_4 hybrids
Per cent			
0			37
0–4.9	6		45
5–9.9	4		6
10–14.9			16
15–19.9			14
20–24.9			9
25–29.9			6
30–34.9			5
35–39.9		2	2
40–44.9		1	3
45–49.9		1	2
50–54.9		2	4
55–59.9		1	4
60–64.9			4
65–69.9			5
70–74.9		1	6
75–79.9		1	6
80–84.9			5
85–89.9			2
90–95		1	1
	—	—	—
Ave. per cent bunt	5	57	21.9
Total No. rows	10	10	182[a]

[a] No count is recorded for one row.

tion of bunt infection percentages for parents and F_4 lines is shown in intervals of 5 per cent bunt infection in table 3.

Of the 183 F_4 progeny rows from 23 F_3 lines, 37 showed 0 per cent of bunt, while an additional 44 fell within the interval, 0 to 5 per cent. Thirty rows showed a greater susceptibility than the average of the Tenmarq parent.

The average per cent of bunt infection for these lines inoculated with a composite of Kansas bunt forms is very similar to that in which form 1 was used, although, as might be expected, the average infection percentages with the composite bunt are slightly higher than those obtained from form 1. The correlation surface and coefficient, $r = 0.8875$, for the 2 sets of data are shown in table 4. In the greater number of lines, infection percentages are low for both form 1 and the composite bunt. Deviations are usually in the direction of lower percentages of infection for form 1 and higher for the composite of Kansas bunt.

It is of interest to note that 30 F_4 lines show zero bunt infection to both form 1 and the composite. An additional 49 rows come within the interval 0 to 5 per cent bunt for both sources of inoculum.

Reaction of Parental Varieties and Hybrids to a Composite of Kansas Bunt Collections, 1933

The bunt percentages of each resistant family as observed in 1932 and 1933 are shown in table 5.

The average per cent of infection in 1933 for Oro is 0.4 and for Tenmarq 33.5, as compared with 5.0 and 57.0 per cent respectively for 1932.

A number of zero and near zero rows were obtained in the F_3 generation, which showed 20 and 13 per cent bunt in the F_2 generation in 1932. On the other hand, bunt infection percentages of several F_3 lines were higher than those of the parent F_2 cultures. This is evidence that these F_2 populations segregated for bunt resistance, with the larger number of segregates showing resistance to bunt.

The F_4 and F_5 hybrid selections, highly resistant in 1932, continued to show very little or no infection in 1933. Since the infection percentages for the parental rows are somewhat lower in 1933, a number of the hybrid rows may later show a small amount of infection during seasons more favorable for the development of the bunt organism.

The percentages of bunt in 1933 from rows showing different degrees of susceptibility to bunt in 1932 are shown in table 6.

Results in the preceding table indicate that most of the rows showing susceptibility to bunt in 1932 are still segregating, as seems evident from the zero and other low bunt-infection percentages. In the selection of smut-free plants for sowing, the more resistant ones evidently were selected, since

TABLE 4.—*Correlation of percentages of bunt infection in F_4 lines of Oro × Tenmarq, inoculated with physiologic form 1 of Tilletia levis and with a composite of Kansas bunt forms*

Composite forms	Physiologic form 1																
	Per cent bunt																
	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	55–59.9	60–64.9	65–69.9	70–74.9	75–79.9	
95–100									1								1
90–94.9												1		1			2
85–89.9						1	1		1				1			1	5
80–84.9									1	2		1		2		1	6
75–79.9									1	2		1		1	1		6
70–74.9					1			1			1		2				5
65–69.9					1					1		1		1			4
60–64.9							1		1		1	1	1				4
55–59.9						1				1	1	1					4
50–54.9										1	1		2				2
45–49.9						1							2				3
40–44.9		1							1								2
35–39.9				1	2	1	1										5
30–34.9	1			2	2	1											6
25–29.9		1	2	2	3			1									9
20–24.9		2	2	3	5		1	1									14
15–19.9	2	4	1	3	4		1	1									16
10–14.9	2		2	2													6
5–9.9	79	3	1														83
0–4.9																	
	84	11	8	13	18	5	5	4	5	7	2	6	6	6	1	2	183

Correlation coefficient r = 0.8875 ± 0.0106.

TABLE 5.—*Distribution of bunt percentage infections of Oro, Tenmarq, and of F_3, F_4 and F_5 hybrids of these varieties, 1933*

TABLE 5.—*Distribution of bunt percentage infections of Oro, Tenmarq, and of F_3, F_4 and F_5 hybrids of these varieties, 1933*

1933 Row No.	Number of rows 1933	Per cent bunt, 1933													Ave.	Per cent bunt 1932
		0	0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60		
Oro	19	15	3		1[a]										0.4	5.0
Tenmarq	20			1[a]		4		3	3	4	2		3		33.5	5.7
F_3 hybrid																
4102	35	12	10	2	5	3	1		1	1					7.0	20.2
4103	19	8	6	2	1			1		1					5.4	13.4
F_4 hybrid																
4106	21	21													0.0	1.1
4107	5	5													0.0	1.3
4112	2	2													0.0	2.2
4117	4	4													0.0	0.0
F_5 hybrid																
4127	5	5													0.0	1.4
4135	5	5													0.0	0.0
4148	1	1													0.0	0.0
4159	1	1													0.0	0.0
4165	4	4													0.0	1.8
4166	2	2													0.0	0.4
4171	2	2													0.0	1.1
4174	2	2													0.0	2.1
4176	3	3													0.0	1.6
4177	6	6													0.0	0.4
4178	3	3													0.0	2.[illegible]
4179	1	1													0.0	1.0
4181	3	3													0.0	0.4
4182	3	3													0.0	0.0
4184	10	10													0.0	0.0
4194	5	5													0.0	0.0
4197	5	5													0.0	0.0
4198	6	6													0.0	2.1
4201	6	5	1												0.3	0.0
4213	1	1													0.0	0.0
4214	2	2													0.0	0.4
4215	5	5													0.0	0.4
4216	6	6													0.0	0.0
4217	5	5													0.0	0.8
4218	7	7													0.0	0.4
4219	6	6													0.0	0.0

TABLE 5.—(*Continued*)

1933 Row No.	Number of rows 1933	Per cent bunt, 1933													Ave.	Per cent bunt 1932
		0	0–5	5–10	10–15	15–25	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60		
4221	8	8													0.0	0.4
4224	5	5													0.0	0.0
4228	3	3													0.0	1.6
4232	3	3													0.0	0.9
4233	2	2													0.0	0.8
4234	2	2													0.0	0.0
4235	3	3													0.0	2.5
4236	3	3													0.0	0.0
4237	3	3													0.0	0.8
4251	5	5													0.0	0.0
4252	3	3													0.0	0.0
4261	3	3													0.0	0.3
4262	3	2	1												0.4	0.0
4263	4	4													0.0	0.7
4264	4	4													0.0	1.8
4265	2	1	1												0.4	1.6
4266	3	3													0.0	0.0
4267	6	6													0.0	0.7
4269	4			3					1						12.4	15.0
4272	6	6													0.0	0.3
4275	3	3													0.0	0.0
4279	3	2	1												1.5	0.0
4281	9	9													0.0	2.2
4282	3	3													0.0	0.8
4283	1	1													0.0	0.0
4284	4	4													0.0	0.0
4285	3	3													0.0	0.0
4301	2	2													0.0	2.0
4302	3	3													0.0	2.4
4303	8	7		1											0.7	0.7
4304	4	4													0.0	0.0
4305	5	5													0.0	1.2
4306	4	4													0.0	2.2
4307	4	4													0.0	2.2
4309	1	1													0.0	0.0
4312	1	1													0.0	0.0
4315	1	1													0.0	0.0
4316	3	3													0.0	0.0
4323	4	3	1												0.2	0.0

[a] These percentages are not included in the average as the total number of culms was too small. The stand was reduced by dry weather and chinch bugs.

TABLE 6.—*Percentages of bunt infection of F_4 and F_5 Oro × Tenmarq hybrids which showed susceptibility to bunt in the F_3 and F_4 generations*

Row No. 1933	Per cent bunt 1932	Per cent bunt 1933
	F_3 lines	F_4 lines
3105	55.0	25.7
3106	49.4	2.8
	F_4 lines	F_5 lines
3113	23.9	0.0
3125	21.5	0.0
3133	23.4	8.7
3137	19.6	5.7
3173	48.0	8.2
3282	45.7	29.8
3271	58.7	39.0
3136	64.3	20.0
3269	91.0	17.0
3281	87.7	5.5
3351	83.8	39.2
3352	86.7	38.6

none of the seed from partially smutted plants was saved. These results are not entirely conclusive, however, as a number of plants from one susceptible row were not planted. It would seem that if pure-line selections were made in these generations for bunt resistance, plants selected at random from such pure lines should produce approximately the same percentages of infection. The variation in the results obtained may be due to unfavorable weather conditions, since the Tenmarq checks did not show a high percentage of infection in every case.

Reaction of Parental Varieties and Hybrids to a Natural Infection of Leaf Rust, 1932

A natural infection of leaf rust occurred on the F_4 hybrids of Oro × Tenmarq grown in 1932.[4] Leaf-rust notes were taken on plants in a foot length of each row in the composite bunt series left standing in the field for this purpose.

The Oro parent is highly susceptible to leaf rust, while Tenmarq is only moderately so. The F_4 lines of Oro × Tenmarq show a wide range of infection with evidence of transgressive segregation. A number of the F_4 lines show very high resistance to leaf rust. The data on leaf-rust infection are summarized in table 7.

A study was made of the relation of percentage of leaf rust and bunt among the F_4 lines of Oro × Tenmarq.

[4] Conditions were very unfavorable for leaf rust infection in 1933.

[a] These percentages are not included in the average as the total number of culms was too small. The stand was reduced by dry weather and chinch bugs.

TABLE 7.—*Distribution of leaf-rust infection of parents and F_4 lines of Oro × Tenmarq, into 5-percent classes*

Per cent of rust	Oro	Tenmarq[a]	F_4 hybrid
5–9.9			16
10–14.9			47
15–19.9			6
20–24.9			20
25–29.9		1	6
30–34.9			
35–39.9			
40–44.9		3	2
45–49.9		3	2
50–54.9			
55–59.9			
60–64.9			
65–69.9	1		9
70–74.9			
75–79.9			
80–84.9	6	2	13
85–89.9	3		28
Ave. per cent rust	80	48.9	37.2
Total No. rows	10	9	139[b]

[a] One row showed segregation for leaf rust.

[b] Rows that were segregating for resistance to leaf rust are not included.

In some F_4 lines the percentages of both leaf rust and bunt are high, while other lines are resistant to both diseases. Some lines are resistant to leaf rust and susceptible to bunt; others show a reciprocal reaction. Selections from F_4 rows resistant to leaf rust and bunt are of particular interest and value.

The correlation coefficient ($r = 0.0151 \pm 0.5760$) indicates the absence of correlation between rust and bunt percentages in these F_4 lines.

DISCUSSION

These studies suggest the presence of several factors in the inheritance of resistance to bunt in the cross, Oro × Tenmarq. The number of zero and near-zero bunt lines observed in F_3 and F_4 progenies indicates that high susceptibility to bunt is recessive in this cross. Although Tenmarq is susceptible, it may carry one or more factors for resistance that differ from those for resistance carried by Oro, the resistant parent.

The number of F_3 lines was far too small to permit a genetic analysis of resistance to bunt in this cross. The behavior of the F_4 lines, therefore, has been used to determine as nearly as possible the manner of inheritance

of bunt resistance. The results suggest that in this cross a number of factors are operative in governing reaction to bunt. There is evidence of the presence of complementary factors governing reaction to bunt. The large number of zero lines suggests that Tenmarq may carry a factor or factors for resistance that, when combined with factors for resistance in Oro, produce greater resistance in some of the hybrids than that expressed by the resistant Oro parent.

The larger number of F_3 and F_4 lines are grouped near the average bunt percentage of the resistant Oro parent, which suggests that in this cross resistance to bunt is partially dominant and that high susceptibility is recessive.

On the basis of F_4 tests, the F_3 lines may be grouped into an 8:12:3 ratio, representing homozygous-resistant, heterozygous, and homozygous-susceptible families, respectively. The number of F_3 lines was small, and this is an abnormal ratio and cannot be given much weight in interpreting the results.

Previous selection on the basis of plant and kernel characters has been practiced. This may have affected the bunt reaction observed in F_4, although, as far as known, the selection was random for bunt resistance. Any association between reaction to bunt and other characters would tend to cause considerable deviation. However, no association between the resistance to bunt and other characters has been observed.

The high correlation between reaction to physiologic form 1 and the composite of bunt forms suggests that possibly there is only one form of bunt of importance in Kansas, as far as the cross Oro × Tenmarq is concerned. However, several rows show considerably greater susceptibility to the composite bunt, and any deviation usually is in the direction of higher bunt infection in the composite series. Physiologic form 1 was represented to a much greater extent than any other form in this composite inoculum, and this form probably caused a large part of the infection. It is possible that only occasionally a row or plant was infected with another form, present in smaller quantity, but sufficiently concentrated to cause infection.

Oro is very susceptible to leaf rust; Tenmarq is only moderately so. Transgressive segregation for resistance to leaf rust indicates that both parents carry factors for resistance to this disease. The plants of both parents were rather uniformly and characteristically infected. Hence it seems evident that the rows with low leaf-rust infection are really resistant and did not merely escape infection. Certain groups of F_4 lines from particular F_3 lines were uniformly resistant to leaf rust; others were susceptible. The inheritance of leaf-rust resistance in the Oro × Tenmarq cross, can probably best be explained on a multiple-factor basis.

SUMMARY

A study of the inheritance of resistance to bunt (*Tilletia levis*) was made in F_2, F_3, and F_4 generations of the cross, Oro × Tenmarq. The resistance to leaf rust (*Puccinia triticina*) was studied in the F_4 generation.

The results indicate that high susceptibility to bunt is recessive in this cross.

F_4 lines were grown that showed greater resistance to bunt than the resistant Oro parent, indicating that the susceptible Tenmarq parent may carry a factor or factors for resistance to bunt.

The inheritance of resistance to bunt in this cross probably is governed by multiple factors.

The correlation coefficient of $r = 0.8875 \pm 0.0106$ between the reaction of the hybrid selections to physiologic form 1 and the reaction of the same selections to a composite of Kansas bunt collections is very high.

Transgressive segregation was noted in a study of leaf-rust reaction, indicating that factors for resistance to leaf rust probably are present in both parents.

No correlation was found between bunt and leaf-rust infection in the Oro × Tenmarq hybrids.

A number of rows in 1932 and 1933 appeared to show a combination of the superior qualities of the Oro and Tenmarq parents and indicate that further selection should yield a bunt-resistant variety of considerable agronomic value.

KANSAS AGRICULTURAL EXPERIMENT STATION
MANHATTAN, KANSAS

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GROWTH OF MICROORGANISMS AT AND BELOW 0° C.

J. A. Berry and C. A. Magoon

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INTRODUCTION

The growth of microorganisms at freezing and subfreezing temperatures is not only of general biological interest but also of considerable practical importance, particularly as it concerns the preservation, storage and handling of foods. With the recent rapid development of the frozen-pack food industry, involving vegetables and fruits, as well as fish and meats, the matter has taken on added significance. In an earlier paper one of the writers (6) reported on studies conducted in these laboratories in which it was shown that the careful selection of the proper storage temperature may determine, to a large extent, the fate of the microorganisms present on the frozen products. Thus, under certain conditions, very moderate cold storage temperatures (−2° and −4° C.) resulted in greater destruction than that occurring at a considerably lower temperature (−20° C.). This emphasizes the need for accurate information on the minimum growth temperatures of the bacteria and other microforms normally present on raw food materials to serve as a guide to the best refrigeration practices for these products. The purpose of the present paper is to record the results of studies along this line on microorganisms derived from frozen-pack fruits and vegetables in these laboratories and to bring together from the literature the reported observations of other workers bearing directly on this subject.

Following the important early observations of Burdon-Sanderson (17) in 1871 that all bacteria are not destroyed by exposure to freezing temperatures, a very considerable amount of work has been done on the low-temperature relations of microorganisms by various research workers in different countries. Since, however, the bulk of this work has been concerned with the destruction-survival phases rather than with the actual growth of the organisms at freezing and subfreezing temperatures, it is beyond the scope of the present paper to review all of it in detail. Suffice it to say that, on the whole, the findings have shown a very marked destructive action of cold, the germicidal effect being particularly noted during the early stages of the refrigerating process, and continuing, though at a diminished rate, for many months. Complete destruction, however, has been attained but rarely, even though extremely low temperatures have been employed (−180° to −190° C., Belli (4); −190° and −252° C., Macfadyen and Rowland (38), Onkiehong (43)). Alternate freezing and

thawing (Brehme (10), Rivers (48), etc.) also have been found ineffective in bringing about complete destruction of microorganisms, and survival after low temperature exposures has been noted among nonsporeformers, as well as those that produce spores, and pathogenic as well as nonpathogenic forms.

The actual multiplication of microorganisms at freezing and subfreezing temperatures has been reported by many workers, and, since their recorded observations are pertinent to the problems now in hand, it is proposed to review briefly such of these as have come to our attention. For the interest it may have from the standpoint of the development of this line of inquiry, the observations will be considered in approximately the order of their publication.

LITERATURE

Forster (27), in 1887, probably was the first to call attention to the growth of bacteria at freezing temperatures when he reported that phosphorescent organisms isolated from fish preserved by cold grew well at 0° C. on various laboratory culture media. In 1892 (28) he further recorded having isolated from various natural waters, foods, wastes, rubbish, and soils a number of organisms that were able to grow at this temperature. He noted also that such organisms were present in great numbers on the surface and in the intestines of freshwater fish and were especially numerous in sea water and on sea fish.

In 1888 Fischer (25) also reported the isolation of 14 different kinds of microorganisms, from harbor water and from soils, which grew at 0° C. These included phosphorescent and also nonluminous species, an unidentified fungus, a coccus, and 7 rod forms, 3 of which were fluorescent species having proteolytic properties. Fischer (26) also was probably the first to note, in 1893, the growth of certain pathogenic bacteria (cholera vibrios) at 0° C.

Conradi and Vogt (21) recorded that a "proteus bacillus," another pathogenic organism, grew slowly at this temperature.

Baur (3) and also Brandt (9), in 1902, noted the slow but positive denitrification of nitrite bouillon by *Bacterium lobatum* at 0° C.

The same year Schmidt-Nielson (50) reported the growth at 0° C. of *Bacillus aquatile fluorescens non-liquefaciens, B. granulosum, B. paracoli gasoformans anindolicum, B. radiatum, B. tarde fluorescens, B. pestis,* and *B. proteus fluorescens,* as well as a strain of *Saccharomyces pastorianus I,* a red Torula, and *Actinomyces ochraceus, A. carneus,* and *A. ochroleucus.*

Müller (42), working with sausage meat, fish, intestinal contents of fish, milk, vegetables, meal, garden soil, and muck, isolated 36 different cultures from these products, all of which grew at 0° C. From these he identified

4 strains of *B. fluorescens liquefaciens,* one of *B. fluorescens non-liquefaciens,* one of *Micrococcus flavus tardigradus* and one of *M. carneus.* Four other unnamed species of bacteria were described. In addition were included in the group a Mucor (*M. mucedo* (?)), a Penicillium (*P. glaucum* (?)), an Oidium, and a Blastomyces. He found that not only were the microorganisms capable of developing at 0° C. widely distributed but their vital manifestations were the same at 0° C. as at the higher temperatures, the difference being merely one of intensity.

Feitel (24) reported studies on denitrifying bacteria isolated from deep sea water in which it was shown that about one-third of the cultures developed slowly at 0.3° to 0° C. *Bacillus actinopelte, B. ornatum,* and *Bacterium balticum* were included in this group.

Klein (35) found a Blastomyces causing brown spots on cold-storage beef to be capable of making slight but distinct growth at 28°–30° F. (−2.22° to −1.11° C.) in 10 days. It showed no growth at 16°–18° F. (−8.89° to −7.78° C.) in this period of time, but the organism was not killed by this treatment, as normal growth appeared in 48 hours when the cultures were brought to ordinary room temperatures.

Tsiklinsky (52), in the journal of one of the French expeditions into the Antarctic regions, noted the presence of numerous microorganisms in the intestines of fishes, as well as in the sea water, although the temperature of the water was −1.5° C.

Richardson (47) stated that certain nonproteolytic bacteria were capable of developing on frozen meat. He did not get growth on frozen meats at temperatures of −9° to −12° C., however, and gave it as his opinion that growth at this temperature would be almost impossible. Emphasis was laid on the point that it is the solid state of the medium and not any specific temperature that is the limiting condition for growth and reproduction, although retardation of growth ensues with lowering of the temperature.

Eustace (22), in work on the storage diseases of apples and peaches, recorded the growth of *Penicillium glaucum* on inoculated apples held at 32° F. (0° C.) for 2 months or more, and decay in 2 weeks, in peaches held at this temperature, because of the activity of brown rot (*Sclerotinia fructigena*). *Alternaria* sp., *Glomerella rufomaculans* (bitter rot), *Sphaeropsis malorum* (black rot), *Cephalothecium roseum* (pink rot) and *Venturia inaequalis* did not cause decay at this temperature.

Pennington (44), in a study of so-called "clean" and "market" milks held at −1.67° to −0.55° C., found very marked increase in numbers of bacteria, even though the milk was semi-solid with ice. In her summary she states: "Bacterial growth at the end of a week, even in the cleanest milk, which contained as low as 300 organisms to the c.c., was pronounced. There was a steady increase in the number of organisms for 5 or 6 weeks, and at

their maximum they numbered hundreds of millions. Occasionally they passed the billion mark per c.c." Certain species of bacteria, such as *Bacillus formosus, B. solitarius,* and *B. Raveneli,* were especially resistant to cold and, in pure culture, frequently were the predominating species, or almost so, at the close of the experiment.

Ravenel, Hastings, and Hammer (45) made studies on the effect of storage at 0° and −9° C. on the bacterial flora of 2 grades of milk, one considered the best obtainable and the other a mixed dairy milk of fair quality. No increase in numbers of bacteria was obtained in the milks held at −9° C. for periods of 160 to 203 days. There was clumping of the casein and fat, an increase in soluble nitrogen, and a decrease in acidity. In the milks held at 0° C., however, there was a marked increase in the bacterial content, which resulted in an increase in acidity, an increase in the percentage of soluble nitrogen, and a decrease in the total nitrogen content, due, probably, to the liberation of free nitrogen.

From a series of studies on bacteria in frozen soils Conn (18, 19, 20) reported a rapid multiplication of bacteria in solidly frozen soils, the increase being due to the development of slow-growing forms distinct from those normally making up the majority of soil organisms during the summer months.

Brown and Smith (16) obtained results supporting those of Conn, just mentioned. They noted further that frozen soils possess much greater ammonifying power than nonfrozen soils, that there was an increase in nitrogen-fixing power in frozen soils, and that the denitrifying power decreased gradually with continuance of the frozen condition.

Massee (40), in studies on *Cladosporium herbarum* as the causal agent in "Black Spot" of beef, shipped in cold storage from Argentina, found that this organism grew slowly at 0° C. Growth below this temperature was appreciable only after one month.

Reed and Reynolds (46), investigating the effect of low temperatures on the growth and activities of bacteria in milk, found that at the temperature of −1° C. *Bacillus putidum, B. aerogenes, B. cyanogenes, B. proteus vulgaris, B. coli, B. subtilis, B. fluorescens liquefaciens, B. prodigiosus, Bacterium lactis acidi, Sarcina lutea, Oidium lactis, Microspira tyrogena,* and *M. citricus* were all able to make growth. Considerable variation was noted in the behavior of different organisms at this temperature; certain of them, for example, *Bact. lactis acidi* and others of the acid-producing group, increased at first but decreased under continued storage at this temperature, while still others, such as *Microspira tyrogena,* grew slowly at first but made greater growth as the storage period was continued. Milk, therefore, that was still low in acid and would have been considered sweet had actually undergone marked chemical change, which affected its quality.

Brooks and Cooley (11), working on the temperature relations of apple-rot fungi, found that *Alternaria* sp., *Botrytis cinerea, Cephalothecium roseum, Neofabraea malicorticis, Penicillium expansum, Sclerotinia cinerea, Sphaeropsis malorum,* and *Volutella fructi* grew in inoculated apples at the temperature of 0° C. *Fusarium radicicola* and *Glomerella cingulata* only, of all the species used, failed to grow at this temperature. In commercial cold-storage experiments on rather immature fruit *Penicillium expansum* continued to develop at 0° C. in the case of delayed storage but was unable to grow at that temperature in the case of immediate storage. The spores of the species of Alternaria, Botrytis, Penicillium, and Sclerotinia had germinated in corn-meal agar at 0° C. at the end of a month but those of species of Aspergillus, Cephalothecium, Fusarium, Glomerella, and Pestalozzia had not. The temperature response was found to be greatly modified by the nature of the medium on which they were grown.

Vanderleck (53), in studies on the bacteria of frozen soils in Quebec, found that bacteria increased rapidly in both frozen and nonfrozen soils when abundant organic material was present for decomposition, but severe frost prevented this development. Bacteria decreased in number when the soil thawed, and increases or decreases in numbers were observed to follow generally the rise or fall in soil moisture. Attention was called to these facts also by Conn (18, 19) and by Brown and Smith (16) mentioned above. In a second paper (54) Vanderleck made the important observation that changes produced by bacteria in frozen soils are dependent on the kinds of enzymes produced by the organisms; that is, evident influence of intracellular enzymes depends upon active growth of the organism. Extracellular enzymes, however, may act in the absence of active growth.

Monvoisin (41), working with the molds occurring on cold-storage meats, reported the finding that molds, normally showing abundant white colonies from spores in 2 days and mature spores in 5 to 6 days at ordinary temperatures, required 2 to 3 months for the appearance of the white colonies and 4 to 5 months for the maturing of the spores at an incubation temperature of −9° C. on the medium he used. Spores failing to show germination at −6° and −9° C. did so on removal to temperatures of 12° to 15° C. in 24 to 36 hours and pursued normal growth. When these same cultures were returned to the low-temperature rooms the growth continued, though slowly. He emphasized the importance of the amount of water vapor in the air as influencing mold growth at various low temperatures. The molds encountered on cold-store meats, in the order of their importance, were: *Thamnidium elegans, Mucor mucedo, Rhizopus* sp., and *Penicillium glaucum.*

Brown and Halversen (15) found that the number of bacteria in frozen soil increased as the temperature became lower and fell with higher tem-

peratures, regardless of the moisture content. They noted also a decrease with thawing.

Vass (55) reported no increase in bacteria in frozen soils and gave the breaking up of clumps caused by freezing as the explanation of the findings of other workers.

Bidault (7), in a study of the molds of frozen meats, found that colonies of *Choetostylum fresenii* and *Hormodendron cladosporioides,* placed in full vegetative condition in a chamber maintained at a temperature of – 10° C., for 2 months, showed slight growth. This was more marked at the end of 2 more months, the temperature being raised to – 8° C. Other molds—*Penicillium crustaceum, Cladosporium herbarum, Botrytis rosea*—grew at temperatures between – 6° and 0° C. He ranged the various microorganisms investigated in the order of their increasing sensitiveness to cold as follows: *Choetostylum fresenii, Hormodendron cladosporioides, Cladosporium herbarum,* different species of Botrytis, *Thamnidium elegans, Penicillium crustaceum,* Torula, and yeast forms. These last continued their development only in the neighborhood of 0° C.

Brooks and Kidd (14) recorded the growth of *Cladosporium herbarum,* the organism associated with "Black Spot" on meats, at temperatures not rising above – 5.5° C. and stated that this was by no means the minimum for this organism. They also mentioned evidence that white molds (species of Sporotrichum and Oospora) could develop at temperatures below the freezing point during prolonged storage.

Stiles (51), in discussing the preservation of foods by freezing, noted that, while many microorganisms cannot withstand temperatures below – 1° or – 2° C., others can live and develop at temperatures considerably lower and the development of all molds is not rendered impossible by storage at temperatures below the freezing point.

Brooks and Hansford (13) found that, of the fungi developing on meat, *Cladosporium herbarum* not only grew but developed fresh spores at 18° F. (– 7.78° C.); *Sporotrichum carnis,* the organism causing white spots on meats, developed slightly at this temperature and appreciably at 28° F. (– 2.22° C.); *Thamnidium* sp. developed at 28° F. and slightly at 18° F. (– 7.78° C.); *Mucor* sp. developed fairly well at 28° F.; and representatives of the genus Penicillium developed appreciably at 28° F. They also found that the spores of *Cladosporium herbarum* germinated at – 6° C. but that subsequent growth was more rapid if germination had taken place during a short period of exposure to ordinary room temperatures. Failure to induce spores of some of the other molds to germinate at – 6° C. was thought due, perhaps, to unfavorable humidity conditions.

Wright (56) observed that when *Mucor mucedo* had been grown on meat at temperatures ranging from – 2° to – 1° C. until the growth was

well established it formed black spots on the meat when placed in cold storage at −12° to −15° C. Similar results were secured with *Penicillium glaucum*, except that the initial temperature of development was about 4° C. He concluded that *Cladosporium herbarum* was only one of a number of molds that might cause black spot on meats and stated that to prevent mold development in cold storage meat should be held at temperatures not higher than −9° C.

Brooks (12), in an attempt to check the work of Wright, just mentioned, grew *Mucor mucedo* on mutton at −1° C. until growth was well established and then stored the meat at −11° C. for 4½ months. No black spots were obtained. Similar results were secured with a species of Penicillium developed at 4° C. and then stored at −11° C. for a like period.

Fay and Olson (23), in a study of the bacteriology of ice-cream manufacture and storage, found that, during the first few days after placing the ice cream in the hardening room or packing it in ice or brine, there was usually a marked decrease in the number of bacteria, a decrease varying from none to 75 per cent in the first 2 days and continuing after the first week at a much slower rate. Frequently, however, after about a month, there was a slow increase in the numbers of bacteria because of growth at the prevailing low temperatures.

Bardach (1) isolated 11 different kinds of nonspore-forming putrefactive bacteria from sewage, sludge, and salt water, which were able to multiply at temperatures of 0° to −2° C. The ordinary life processes were carried on in a normal manner at these low temperatures, though the rate of growth was slower than at higher temperatures.

Rubentschik (49) found that *Urobacillus psychrocartericus* and *Urosarcina psychrocartericus* were able to grow at temperatures of −1.25° to −2.5° C. In bouillon containing 3 per cent of urea the latter organism increased from an average of 2,870 to 28,520,000 per 1 c.c. in 25 days.

Haines (29, 30, 31, 32, 33, 34), working on some of the organisms developing under cold-storage conditions, found that *Sporotrichum carnis* grew at temperatures of −5° and −7° C. The lower limit of growth of this fungus on supercooled Czapek's agar was near −10° C. Growth was never obtained on the frozen medium. He noted further that, under favorable conditions, special types of organisms could multiply at 0° C. to such an extent as to cause the spoilage of carcasses in storage. His findings indicated that "storage at −5° C. leads to a steady fall in the numbers of bacteria, followed by a period when the numbers are at a minimum and that this in turn is followed by an increase in numbers, due mainly to the growth of yeasts and moulds." In no case did he observe visible growth of bacteria, yeasts, or molds on carcasses of mutton stored at −10° C. or lower. Organisms of the *Pseudomonas* (*fluorescens*) group were found to

grow relatively well at 0° C. Experiments with various strains of Actinomyces showed a minimum growth temperature of 0° C. for some, while one strain grew at temperatures down to about −5° C. Various yeasts and Torulae, commonly present on cold-storage meat, grew comparatively well at −5° C.

Bonney (8), in the discussion of findings from experiments on frozen-pack fruits, conducted cooperatively with workers of the Bureau of Plant Industry, noted that a temperature of 30° F. permitted mold growth and that, under certain conditions, growth might take place also at considerably lower temperatures. The strength of the syrup used in the pack and the degree of vacuum within the container were found to be important growth-inhibiting factors under these conditions.

Lea (36, 37) reported that a storage temperature of −5° C. failed to prevent the growth of molds and yeasts on frozen mutton and lamb, but no visible growth occurred at −10° or −20° C. Freshly slaughtered beef stored at 0° C. and examined at intervals over a period of 42 days showed at the end of the storage period extensive mold growth, while with like samples handled in the same way, but with the air agitated by means of fans, the growth was much retarded and most of the surface was essentially free from visible growth. A carcass of lamb and a side of pork, stored under these same conditions with aeration, showed no appreciable growth of molds or yeasts after 60 days. Molds, yeasts, and bacteria developed on beef held at −1.6° C. for 42 days without air circulation.

Magoon (39) reported marked growth of many molds on apparently solidly congealed frozen-pack fruits and the production of spores by these organisms at temperatures well below 0° C. (commercial storage at about −5° C.); and Berry (5) has recorded the development of pink yeasts and molds in berries packed in paper containers and stored at 28° F. (−2.22° C.).

Because of the widespread interest in the significance of *Clostridium botulinum*, as concerned with the preservation of food products, it may not be amiss to state here that in the review of the work of these numerous investigators no published record has been found of the development of this organism at temperatures below 31° F., as reported earlier by the senior writer (5).

EXPERIMENTAL DATA

In the course of the present work on fruits and vegetables preserved at low temperatures specific information has been secured regarding the growth of a number of microorganisms at temperatures of −4° and −6.7° C. Details as to the types of organisms concerned, the products attacked, and the time periods required for marked spoilage to occur are here briefly presented.

Microorganisms Growing at −4° C.

1. *Pseudomonas fluorescens.* Scalded or "blanched" peas, packed with 3 per cent NaCl solution in nonairtight containers and held at −4° C., showed microbial spoilage after 4 weeks of storage. Ice formation in the product had been slow. The predominating type of organism obtained by plating on nutrient agar in high dilution and incubating at 20° C. was inoculated into sterile pea broth and nutrient gelatin, which were at once put at −4° C. for incubation. In 4 weeks unmistakable growth had taken place. On examination the organisms proved to be Gram-negative rods of medium size, nonspore-forming, motile by means of a single polar flagellum, aerobic, liquefying gelatine rapidly, reducing nitrates, not producing indol nor coagulating milk, and forming a green pigment that diffused into the medium.

The growth curve at −4° C. of this strain of *Pseudomonas fluorescens* in 3 per cent NaCl bouillon, isolated from frozen-pack peas, is shown in figure 1.

2. *Lactobacillus sp.* Peas, prepared and stored as above, except that hermetically sealed containers were used, showed after 8 weeks an increase

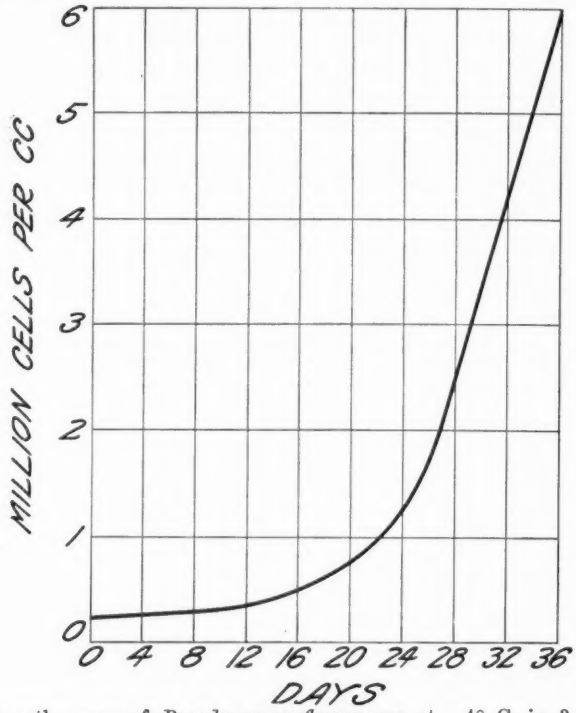

FIG. 1. Growth curve of *Pseudomonas fluorescens* at −4° C. in 3 per cent NaCl bouillon. It was isolated from frozen-pack peas.

in bacterial content, the plates showing unusual numbers of small glistening colonies. The pH of the peas had changed from 6.5 to 6.0. As transfers from these colonies grew in gelatin a series of stab inoculations was made in this medium and the tubes incubated at −4° C. Slight growth was evident after 6 weeks. Study of the organisms showed them to be small blunt rods, Gram-positive, nonspore-forming, microaerophilic, growing slowly on agar and gelatin, which they did not liquefy, not forming indol nor reducing nitrates, forming acid but not gas from sucrose, maltose, dextrose, lactose, and less acid with no gas from mannitol, raffinose, and trehalose.

3. *Torula sp.* Spoilage of strawberries, packed in 50 per cent sucrose solution in nonairtight containers and held at −4° C., was noted after 4 weeks' storage, cultures in nutrient agar at this time yielding pink colonies, previously little in evidence. Inoculations from these were made to 2 per cent nutrient agar by the stab method, and growth was evident at the surface of the medium after 2 weeks' incubation at −4° C. The organisms were mostly oval, about $3 \times 5\ \mu$, reproduced by budding, rapidly formed pink moist growth on nutrient agar at 18–32° C., and did not ferment sucrose, maltose, dextrose, or lactose.

4. *Monilia sp.* In addition to Torulae, whitish patches appeared on the surface of strawberries held for 6 weeks at −4° C. Direct examination showed oval and elongated cells with some filaments. Pure cultures having been secured by plating on wort agar, inoculations were made to tubes of wort, 15° Brix, which were incubated at −4° C. A pellicle formed in 8 weeks. A description of the organism follows: Cells oval, measuring about $3 \times 4.5\ \mu$, with some filamentous forms on nutrient agar, agar colonies small, circular, homogeneous, white, convex; numerous calcium oxalate crystals in the medium; wort-agar colonies about 4 mm. in diameter, mycelioid, convex, not becoming colored; hyphae septate; conidia in clusters at the sides and tips of branched conidiophores. Young wort cultures impart a fruity odor. Acid without gas is formed from sucrose, maltose, dextrose, and levulose but not from lactose.

5. *Penicillium spp.* Mold growth was observed on the outside of several paper containers of peas packed in 3 per cent NaCl solution after 3 months' storage at −4° C. In 7 months the colonies were about 6 mm. in diameter and of a slightly greenish hue. When the containers were moved to the laboratory, the colonies—obviously species of Penicillium—matured in 5 days, appearing flat with powdery surfaces. Most of them were bluish-green—Type I; a few were lighter green—Type II. Some characteristics of these organisms follow:

Type I. Wort-agar colonies, spreading, 2 to 2.5 cm. in diameter, and 1 to 2 mm. in elevation, bluish-green, surface granular, margin zonate, re-

verse salmon orange, odor pronounced; conidiophores in part as short branches of trailing aerial hyphae, but mainly in fascicles; conidial chains 100 to 200 μ long; conidia elliptical, smooth, 3 × 3.5 μ in dimensions. Gelatin not liquefied in 21 days. An inoculation of Type I, mostly spores, on deep wort agar, showed slight growth at −4° C. in 50 days.

Type II. Same as Type I, except that the color is greener, with reverse of the colony yellow green, and gelatin liquefied in 10 days.

Both types readily produced rot in apples. Coremia of the massive type were not noted. The two strains presumably below in the *Fasciculata* section of the genus Penicillium, and may tentatively be placed in the *P. expansum* series.

Microorganisms Growing at −6.7° C.

1. *Cladosporium sp.* Okra, packed in 3 per cent NaCl solution, and kale, without packing liquid and stored in nonairtight containers at −6.7° C. for 15 months and 16 months, respectively, showed, on the surface, numerous dirty white to olivaceous mold colonies, 3 to 4 mm. in diameter. In addition to direct examination, a series of inoculations was made at once on nutrient agar and wort agar. The colonies resulting after a week's incubation at 20° C. appeared to be of a single species. A brief description follows: Colonies spreading, flat, olivaceous, reverse black; hyphae septate, intricately branched, sooty, nodose on nutrient agar; conidiophores branched; conidia terminal and lateral, ovoid, greenish, 3.8 to 6 × 6 to 14 μ; the larger often bicellular.

2. *Sporotrichum sp.* Scalded peas, packed in 3 per cent NaCl solution and stored at −6.7° C. in nonairtight containers, showed, in 10 months, numerous mold colonies on peas that projected above the frozen-pack liquor. The colonies were white, about 3 mm. in diameter, and raised to convex. Mycelial tufts were at once inoculated to nutrient agar, wort agar, and sterile peas. Equally good growth occurred in all 3 types of media in 7 days at 20° C. Following is a brief description:

Colonies, up to 1 cm. in diameter, white at first, later somewhat fawn, about 2 mm. in height; reverse yellow; hyphae septate, conidiophores short, branched, not in whorls; conidia smooth, pyriform, 3.0 × 4.5 μ, not in chains, and borne laterally and terminally on the conidiophores.

Sterile peas in 3 per cent sodium chloride solution inoculated with small mycelial tufts and spores of the *Cladosporium* sp. and *Sporotrichum* sp. mentioned above and placed at once in storage at −6.7° C. showed growth in 3 months. After 4 months' incubation growth was no longer confined to the peas projecting above the brine but had definitely started on the surface of the frozen brine itself.

Media for Cultures at Subfreezing Temperatures

Since ice may be more inimical to the growth of microorganisms than the cold producing it, media intended for cultural studies below 0° C. should remain ice-free at the temperature selected. While, as might be expected, standard bouillon and agar resist physical change at the freezing point of water, experience shows that they are unreliable for long incubation at temperatures near −2° C. For studies at −4° C. it is necessary to depress the freezing point of the bouillon or agar by the addition of an adequate amount of nontoxic solute, such as 3 per cent NaCl. The age and condition of storage of the medium are modifying factors; thus, tubed nutrient gelatin, concentrated by 5 weeks' storage at 18° C. in a relatively dry room, has been used successfully at −4° C. As a corollary, any increase in the percentage of the ingredients used in a medium tends to lower the freezing point. In this connection wort,[1] 12° Brix, has not been found reliable at −4° C.; but, as this medium is used for mold and yeast culture, a wort of 15° Brix, which, as a rule, does not freeze at −4° C., may be employed without inhibitive plasmolytic action on the cells.

DISCUSSION

This brief survey of investigations already made shows that freezing temperatures can by no means be relied upon to prevent the microbial spoilage of food products when those temperatures are at or even well below zero Centigrade. The organisms able to carry on at these temperatures are widely distributed and of various kinds. In the studies here reviewed no less than 15 different genera of fungi and more than 30 different species of bacteria, as well as yeasts and their close relatives, the torulae, have been found able to grow and function normally under these low-temperature conditions. To be sure, in most cases this growth has been relatively slow, but when considered in relation to practical refrigeration practices in which storage for long periods is common, it becomes of very real significance. It is of particular value to know that the complete life cycle of some food-destroying fungi, from spore germination through the vegetative phases of growth to complete spore production, may take place at temperatures as low as −7.78° C., that spore production may take place at −9° C., and that vegetative growth and food deterioration induced thereby may proceed at a temperature of −10° C. or, possibly, even somewhat below this point.

The physical condition of the refrigerated material and the environmental factors seem to be more important than the degree of cold in de-

[1] This useful medium is readily prepared from commercial malt syrup. The unadjusted reaction is approximately pH 4.6.

termining whether or not growth will take place. The mere presence of ice in the product is no criterion of thorough preservation. In the case of molds, at least, the presence or absence of free oxygen and, to a considerable degree, the amount of moisture available are important determining factors. The favorable effect of sugar or sugar syrup in frozen-pack fruits in preventing mold growth probably is due to its high osmotic or dehydrating action.

It is apparent that under the conditions of moderate cold, *e.g.*, $-7°$ to $-2°$ C., the majority of microorganisms are killed but that a few survivors will remain that, in the absence of inhibiting conditions, are able to develop. These cold-tolerant organisms may or may not be prominent in the original microflora. Incidentally, this adaptability of microorganisms to low-temperature conditions has its parallel in the ability of certain individuals, not true thermophiles, in many microbial populations to adapt themselves to relatively high temperature conditions. It would appear from the data reviewed that the lower temperature limit for growth lies close to $-10°$ C., though the report of Bonney (8) suggests that under certain conditions the minimum temperature may be even lower than this.

It is important to note that among these organisms recorded as growing at zero Centigrade are included some of the pathogenic forms.

The direct findings of Brooks and Cooley (11) to the effect that mold growth that is prevented by prompt refrigeration may proceed at the same storage temperature if refrigeration be delayed, together with the findings of other workers (41, 13, etc.) that vegetative growth of some molds may proceed at temperatures that prevent the germination of their spores, and the observation (54) that enzymes continue to function after the growth of the cells that produced them has been inhibited by cold, all emphasize the practical importance of prompt handling and refrigeration of those products intended for frozen-pack storage. Such prompt handling and storage add materially to the chances for successful preservation.

The increase in the numbers of organisms in frozen soils, attributed by several workers to the development of organisms especially adaptable to low-temperature conditions, might be due, it would seem, to changes in the food material—the formation of ice resulting, perhaps, in a more favorable concentration of the nutrients or in alteration in its composition. The findings of Barnes (2) that the growth and photosynthesis of Spirogyra are greatly accelerated and the multiplication of Protozoa is more rapid in cultures rich in trihydrol, that molecular form of water particularly abundant in freshly melted ice, are very suggestive as bearing on this point. The possibility of lessened antibiosis should also be considered in this connection.

It is of interest to note that of the various microorganisms growing at

or below 0° C. none thus far investigated can properly be classed as "psychrophiles." Müller (42), among others, has called special attention to the fact that this designation is a misnomer, and the findings in the present work confirm his conclusions; for while many organisms are able to grow at these low temperatures, it is apparent that the growth conditions are not optimal, as all develop more rapidly at temperatures well above the freezing point. They appear, therefore, to be a group of mesophilic organisms that are able to carry on relatively slowly at a lower temperature range than the majority of microforms.

SUMMARY

A review of the literature on the minimum temperature requirements of microorganisms shows that:

Since 1887, numerous species, including even some of the pathogenic forms, have been observed to grow at 0° C. and, in some cases, considerably below that temperature. In general, the organisms developing at subzero temperatures are fungal rather than bacterial, though some bacterial growth has been observed.

Of those organisms developing at temperatures below −5° C. species of Cladosporium and Sporotrichum have been most commonly observed.

In general, following exposure to moderately low temperatures, *e.g.*, −5° to −6° C., there is a marked reduction in the number of viable organisms. Under aerobic conditions, however, this initial decrease may be followed by an actual increase of cold-tolerant molds.

There is evidence that the development of molds may take place at temperatures below those at which their spores can germinate. Spore germination of certain molds, however, has been observed at temperatures as low as −7.78° C.

Any microbial growth below −10° C. seems unlikely.

Failure of an organism to grow at a subzero temperature may be due to ice formation in the medium rather than to the effect of cold alone, though apparently some microforms, particularly certain molds, are less affected by ice formation than others.

Humidity and oxygen relationships are factors to be evaluated in a consideration of microbial growth at low temperatures.

No reported record has been found of the growth of *Clostridium botulinum* at subfreezing temperatures.

The writers' records show growth at −4° C. of *Pseudomonas fluorescens* and species of Lactobacillus, Torula, Monilia, and Penicillium. Growth of species of Cladosporium and Sporotrichum at −6.7° C., also, is reported. These organisms were isolated from frozen-pack fruits and vegetables and grown in pure cultures at the temperatures mentioned.

A graph shows the rate of growth of a strain of *Pseudomonas fluorescens* at −4° C.

Types of culture media suitable for use in the study of microbial growth at temperatures below 0° C. are considered.

The contention that a truly cold-loving or "psychrophilic" group of microorganisms exists appears unwarranted by available evidence. While organisms recorded as growing at 0° C. or below may with propriety be regarded as forming a cold-tolerant group, their temperature requirements for best growth definitely place them in the mesophilic class.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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THE COMPARATIVE BEHAVIOR OF FOUR CLOVER-LEAF PARASITES ON EXCISED LEAVES[1]

CECIL E. YARWOOD[2]

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INTRODUCTION

In the course of inoculations with a number of clover pathogens on excised leaves it was observed that incubation conditions favorable to some fungi were unsuitable for the development of others. One important factor, as shown in this paper, was found to be the nutritional condition of the excised leaves.

The literature, prior to 1924, on the use of excised leaves for infection experiments with parasitic fungi, especially the rusts, has been reviewed by Clinton and McCormick (1). Mains (3) was perhaps the first to show the value of a sugar nutrient solution in such studies. Waters (7) controlled the formation of urediniospores and teliospores of a number of rust fungi by manipulating the carbohydrate supply of excised leaves. Trelease and Trelease (6) used excised leaves in demonstrating the importance of available sugars for the development of wheat mildew. Of a number of sugars tested Waters found sucrose the most suitable, but Trelease and Trelease found sucrose, dextrose, and glycerine about equally valuable.

Salmon (5) was perhaps the first to use excised clover leaves for infection experiments. He inoculated such leaves with powdery mildew in moist Petri-dish chambers and reported successful infection. Clinton and McCormick inoculated excised clover leaves, floating on water, with rust and they state that, "With such tender leaves as clovers, however, death of the leaves often occurred too early to secure definite results." Waters secured satisfactory infection with rust on excised leaves of alsike clover.

MATERIALS AND METHODS

Four foliage diseases of red clover, *Trifolium pratense* L., were studied on excised leaves, namely: rust caused by *Uromyces fallens* (Desm.) Kern., mildew caused by *Erysiphe polygoni* DC., leaf spot caused by *Macrosporium sarcinaeforme* Cav., and anthracnose caused by *Colletotrichum trifolii* S. M. Bain. The first two of these diseases are caused by obligate parasites

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and the latter two by facultative parasites. Pathogenicity studies with *Cicinnobolus cesatii* (syn. *Ampelomyces quisqualis*), the common parasite of powdery mildews, have also been conducted on excised mildew-infected clover leaves.

Spores of Uromyces and Erysiphe, to be used in inoculations, were produced on living clover leaflets floated on a 10 per cent sucrose solution, while those of Colletotrichum, Macrosporium, and Cicinnobolus were produced in agar cultures. Leaflets to be inoculated were excised from disease-free plants and floated on sucrose solution or water in Syracuse watch glasses or Petri dishes.

The inoculation of the leaflets with Erysiphe was made by touching their upper (ventral) surfaces with another mildewed leaflet and the inoculated leaflets were floated with their lower surfaces resting on the nutrient solution and with their inoculated surfaces upward. Spores of Cicinnobolus were atomized in suspension on mildewed or mildew-inoculated leaf surfaces. Uromyces spores were dusted on the lower leaf surface, the leaflets atomized with water and floated with their upper surfaces resting on the solution. Spores of Colletotrichum and Macrosporium were atomized over the lower leaf surface in water suspension and the leaflets were floated with their upper surfaces resting on the solution.

In most of the experiments reported here the 3 leaflets of a single leaf were compared with one another, each leaflet being exposed to a different treatment. By placing each of the 3 leaflets of a number of leaves into separate dishes, 3 closely comparable units of unlimited size could be secured. Each leaflet was numbered with ink when its identity was desired. This method of comparison is similar to the "twin leaf method" of Denney (2) and will be referred to as the "triplet leaflet method." All inoculated and control leaflets were incubated at 17° to 21° C. in diffuse light, unless otherwise stated.

The observations and photographs presented usually were made about 12 days after inoculation. The incubation period, however, varied with different plants, with different parasites, and different treatments. Under optimum conditions for development, the Colletotrichum and Cicinnobolus normally sporulate about 5 days after inoculation, the Erysiphe in about 6 days, the Macrosporium in about 8 days, and the Uromyces in about 10 days. Injury from Colletotrichum and Macrosporium was generally evident before sporulation and the leaflets frequently died and decayed before spores were formed.

In all cases leaves from four or more plants were used in the experiments reported. The results and illustrations presented are to be considered of relative value only, as there was considerable variation in the results with different leaves and with different plants.

THE EFFECT OF DIFFERENT NUTRIENT SOLUTIONS ON THE VITALITY OF EXCISED CLOVER LEAFLETS

Before attempting inoculation experiments with the clover pathogens, it was necessary to determine the best nutrient solution on which to float the excised leaflets. Nonsterilized solution of commercial cane sugar (sucrose) was found most suitable for excised leaflets, thus bearing out the findings of Waters (7). The organic nutrients, sucrose, glucose, maltose, and asparagin, and the inorganic nutrients, $Mg(NO_3)_2$, $CaH_4(PO_4)_2$, and K_2SO_4 were also used alone and in different combinations as nutrients. Dextrose was better than maltose but both allowed a greater growth of contaminating fungi and bacteria than did sucrose. The mineral nutrients, as compared with distilled water, did not appreciably increase the life of the excised leaflets. Magnesium nitrate especially favored bacterial growth and $CaH_4(PO_4)_2$ favored the growth of fungus contaminants.

Solutions, varying from 1 to 50 per cent of dissolved cane sugar, were compared as nutrients. The vigor of the excised leaflets increased with the concentration of the solution up to about 16 per cent sucrose. Healthy clover leaflets floating on distilled water usually lived 1 to 3 weeks. Leaflets on 10 per cent sucrose normally remained in good condition from 2 to 6 weeks and have been kept living for 17 weeks. The maximum concentration of sucrose on which the leaflets remained in good condition was about 32 per cent.

With age the cultures always became contaminated but contamination was not an important factor in these experiments. Cleaned but generally nonsterilized glassware was used and no attempt was made to secure aseptic leaves nor to sterilize the nutrient solutions. The cultures usually remained free of apparent contamination until the edges of some of the leaves began to decay.

CULTURES OF UROMYCES, ERYSIPHE, MACROSPORIUM, AND COLLETOTRICHUM

Under field conditions these 4 parasites may be found simultaneously, but in dish cultures they show marked differences in response to the physiological condition of the leaves. In dish cultures a given leaflet generally was inoculated with only one parasite; in some cases, however, the 4 parasites were inoculated simultaneously on the same leaflet. Both methods gave essentially the same results but the former method was more satisfactory, since it was difficult to distinguish the effects of different fungi when all were inoculated on the same leaflets.

The leaflets and the condition surrounding them were modified in three ways: by floating them on solutions of varying nutrient value, by removing them at different times of the day, and by selecting leaves of different ages.

Three nutrient solutions, namely, water, 2 per cent sucrose, and 10 per cent sucrose, were found very satisfactory for determining the response differences of these pathogens to the nutritional condition of the leaflets.

Effect of Nutrient Solution

Representative results of a test to determine the effect of the nutrient condition of the leaflets on the development of these parasitic fungi are illustrated in figure 1. Although infection resulted, leaflets inoculated

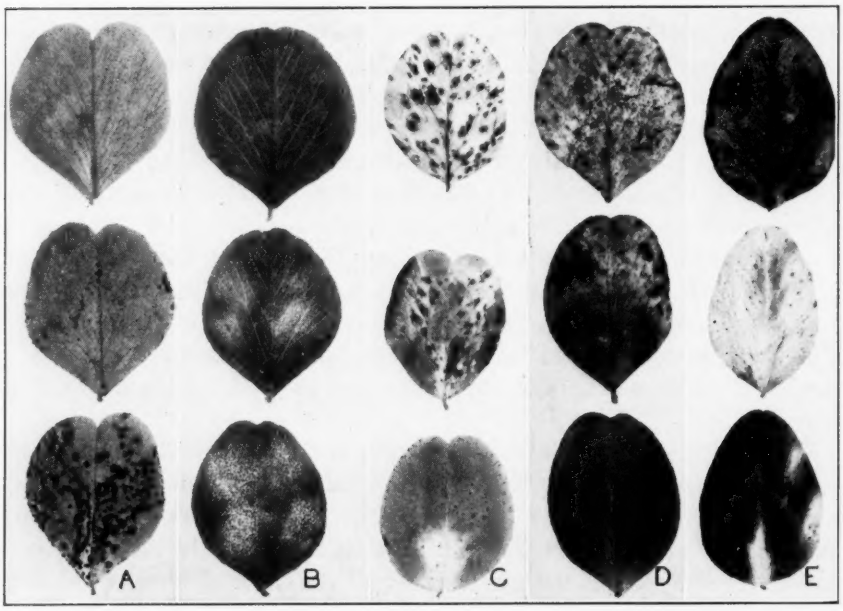

FIG. 1. Effect of sucrose nutrient solutions on the susceptibility of clover leaves to parasites. Each vertical row of leaflets is from the same leaf. Two different plants are represented. The leaflets of the upper row were floated on water, those of the center on 2 per cent sucrose, and those of the lower on 10 per cent sucrose. A. 16 days after inoculation with Uromyces. B. 12 days after inoculation with Erysiphe. The leaflets are somewhat atypical in that leaflets inoculated with mildew and floated on water normally show considerable chlorosis. C. 11 days after inoculation with Macrosporium. D. 14 days after inoculation with Colletotrichum. E. 14 days after removing from the plant but not inoculated.

with Uromyces showed little if any injury on any of the solutions. The optimum concentration of sucrose solution for rust sporulation was about 10 per cent; maximum, about 16 per cent. In most cases there was very little sporulation on leaflets on 2 per cent sucrose, and none on leaflets on water.

Compared with controls, leaflets inoculated with Erysiphe and floated

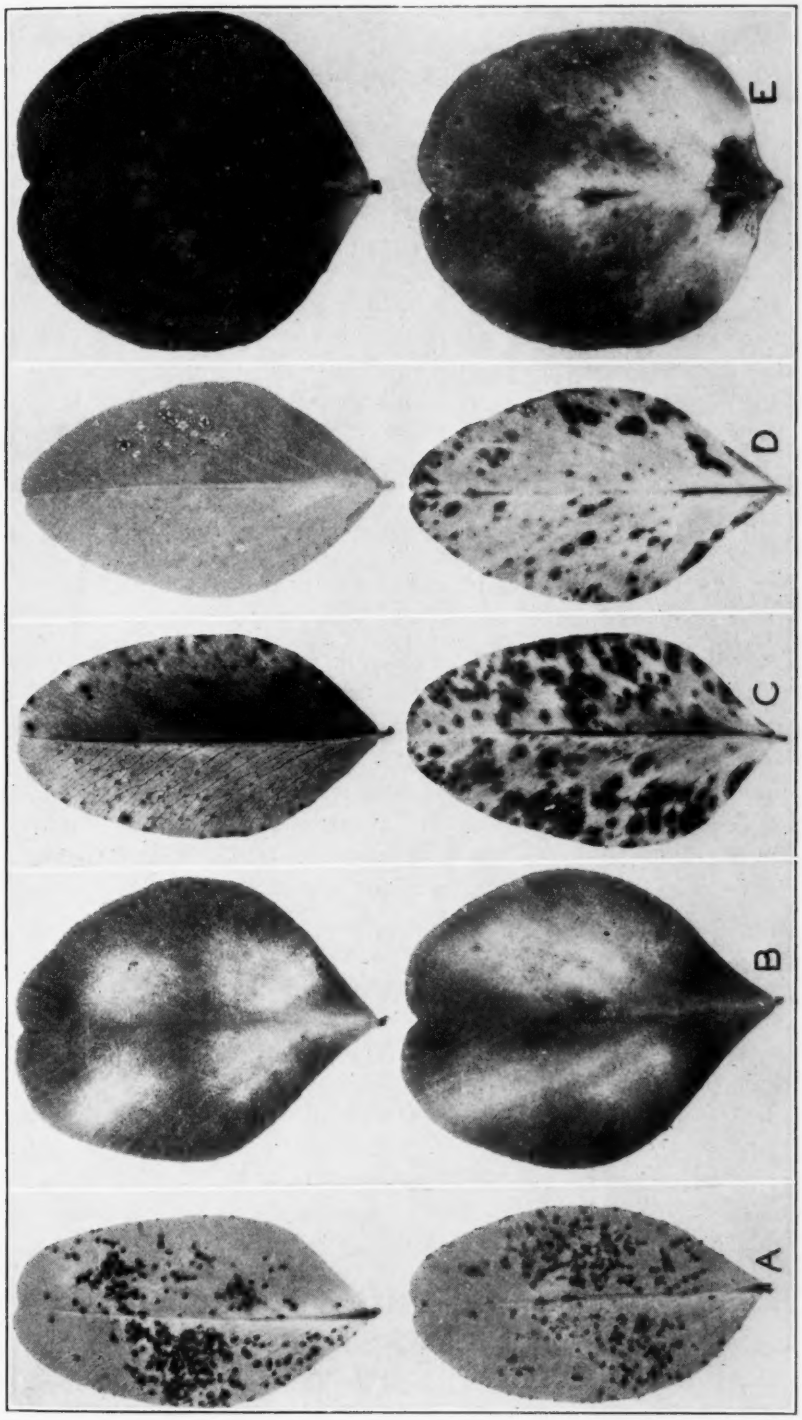

FIG. 2. Effect of the time of removing leaflets from the plant on their susceptibility to parasites. Each vertical pair of leaflets is from the same leaf. Different plants are represented. The leaflets of the upper row were removed at 5 p.m. and those of the lower row on the following morning at 7 a.m. A. 13 days after inoculation with Uromyces. B. 10 days after inoculation with Erysiphe. C. 7 days after inoculation with Macrosporium. D. 10 days after inoculation with Colletotrichum. E. 12 days after removing from plant but not inoculated. Leaflets in A and B floated on 10 per cent sucrose; in C to E on water.

on water were generally severely injured. The injury decreased as the sucrose concentration of the medium was increased up to 16 per cent. The sporulation of Erysiphe was greatest on leaflets floating on 16 per cent sucrose and decreased with increasing or decreasing sucrose concentrations; but the optimum range was not so clearly defined as for Uromyces infection.

Leaflets inoculated with Macrosporium were severely injured when floated on water, less so on 2 per cent sucrose, and least on 10 per cent sucrose (Fig. 1, C). Macrosporium sporulated very little on leaflets on water but fairly abundantly on leaflets on 2 per cent sucrose. Leaflets on 10 per cent sucrose were apparently very resistant at first but, as the leaflets aged, the fungus sporulated heavily on the dead and dying parts.

Colletotrichum caused the greatest injury to leaflets on water and least to leaflets on 10 per cent sucrose (Fig. 1, D). The greatest initial sporulation was coincident with the greatest injury to the leaflets, but, later, the greatest sporulation occurred on leaflets on 2 per cent sucrose. No sporulation of Colletotrichum was observed on leaflets on 10 per cent sucrose.

THE EFFECT OF THE TIME OF DAY OF REMOVING THE LEAVES FROM THE PLANT ON SUSCEPTIBILITY TO AND DAMAGE FROM INFECTION

Several workers, among them Miller and others (4), have shown that, due to photosynthesis, the carbohydrate and dry matter contents of green leaves normally increase during the day, reach a maximum in the afternoon, and decrease to a minimum during the night and early morning. Since it appears that the carbohydrate content of the leaves is a factor affecting their susceptibility to disease, as was indicated by the results with sucrose nutrient solutions, it may be reasoned that the time of day when the leaves were removed from the plant, before being inoculated in dish cultures, would affect their susceptibility to disease.

Experiments to investigate this possible relationship were performed in March and April. Two times of removal, one at about each extreme of the normal diurnal variation in the carbohydrate content of the leaves, were found most satisfactory for demonstrating the effect of time of removal of leaves on their vigor and on their resultant susceptibility to disease. Each leaflet of a leaf was removed from the plant at a different time but all were inoculated at the same time and subjected to subsequently similar conditions. One leaflet (A) of each leaf tested was removed at 5 p.m. of a clear day. The leaflets removed were placed in the dark on a nutrient solution and the plants from which they were removed were placed in the dark. Another leaflet (B) from each of the same leaves was removed from the plant the following day at 7 a.m. and also placed on nutrient solution in the dark. The growing plants were then returned to the light. The third leaflet (C)

was removed at 5 p.m. of the second day. All leaflets were inoculated simultaaneously, placed in the dark, and examined after about 5 days.

For these experiments the uninoculated leaflets, the leaflets inoculated with anthracnose, and the leaflets inoculated with leaf spot were floated on water during the incubation period. Because of the poor development of mildew and rust, which resulted when the host leaflets were floated on water or on weak sucrose solution, these leaflets were placed on 10 per cent sucrose.

After several days on distilled water the noninoculated leaflets excised from the plants in the late afternoon were more vigorous than similarly treated leaflets that had been excised the following morning (Fig. 2, E).

The differences in susceptibility of leaflets removed from the plant at different times were most clearly evident on leaflets inoculated with anthracnose. Leaflets removed at 5 p.m. were more resistant than leaflets from the same leaves removed at 7 a.m. the following morning (Fig. 2, D). Similar results were secured by removing leaflets from the plant at 4 different times of the day. These leaflets were taken at 6 a.m., noon, 6 p.m., and midnight. In this comparison the leaflets taken in the late afternoon (6 p.m.) were again the most vigorous and most resistant, while those taken at 6 a.m. were the least vigorous and the most susceptible. The leaflets taken at noon and midnight were intermediate between these extremes of vigor and susceptibility, those taken at noon being slightly more vigorous and resistant than those taken at midnight.

The different times of leaflet removal affected the relative susceptibility to leaf spot in the same way as it did the susceptibility to anthracnose (Fig. 2, C), although the differences were less marked.

Noninoculated leaflets on 10 per cent sucrose generally showed indistinctly any differences in the vigor of leaflets due to the time they were removed from the plant. No distinct differences were found when such leaflets were inoculated with mildew and rust (Fig. 2, A and 2, B), although in some cases a greater sporulation of rust and mildew occurred on the leaflets removed in the late afternoon. It is probable that reducing the concentration of sucrose would have made the original differences in these leaflets more apparent when tested for susceptibility to mildew and rust.

To determine the effect of the age of clover leaves on their susceptibility to disease in dish cultures, leaves of different ages from the same plant were inoculated simultaneously with the same fungus. Young leaves that had just unfolded, leaves of medium age, and the oldest leaves available and still in good condition were selected for the comparison of the effect of the three degrees of leaf maturity. In these tests leaflets inoculated with rust or mildew were floated on 10 per cent sucrose; with leaf spot on 2 per cent sucrose, and with anthracnose on water.

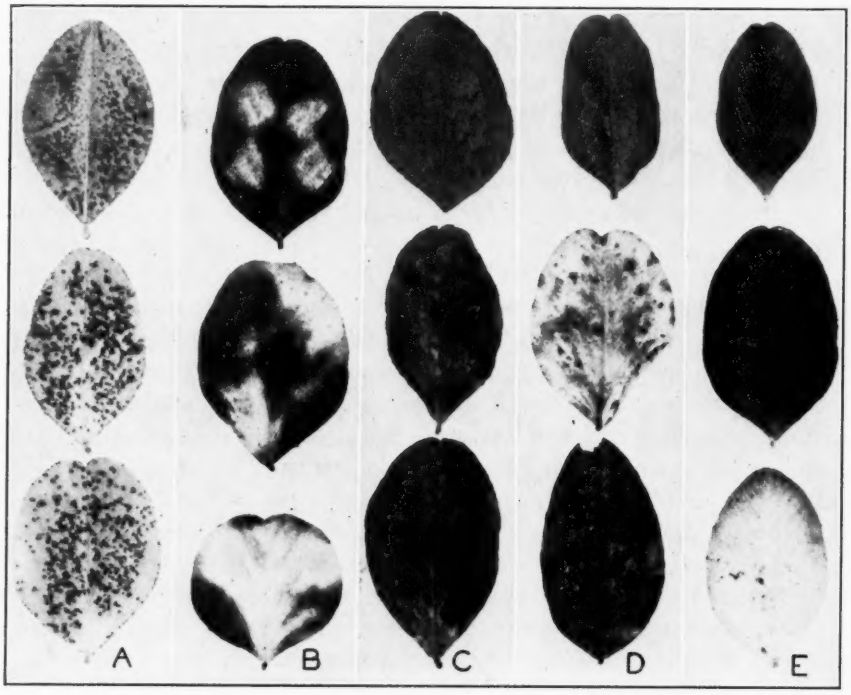

FIG. 3. Effect of age on susceptibility of leaves to parasites. Each vertical row of leaflets is from the same plant, the upper from a young leaf, the center one from a leaf of medium age, and the lower one from an old leaf. A. 16 days after inoculation with Uromyces. B. 10 days after inoculation with Erysiphe. C. 9 days after inoculation with Macrosporium. D. 12 days after inoculation with Colletotrichum. E. 12 days after removal from the plant but not not inoculated. Leaflets in A and B floated on 10 per cent sucrose; in C on 2 per cent sucrose; in D and E on water.

The vigor of the leaflets when floated on 10 per cent sucrose or water, as measured by their ability to remain in good condition for the period of the experiments, definitely decreased with the age of the leaves selected. Young leaves were generally more vigorous than old leaves (Fig. 3, E).

The rust development on young, medium-age, and old leaflets was about the same (Fig. 3, A). With few exceptions mildew grew more vigorously on young and medium-age leaflets than on old leaflets (Fig. 3, B).

Leaf spot and anthracnose, on the other hand, made a more luxuriant development and caused greater injury on old than on young leaflets (Fig. 3, C and D).

CULTURES OF CICINNOBOLUS CESATII DEBARY

Excised clover leaflets in dish cultures have been found very convenient for inoculations with Cicinnobolus. The leaflets were first inoculated with

Erysiphe and immediately after or at a desired later interval were inoculated with Cicinnobolus. Pycnidia of Cicinnobolus were formed in the conidiophores of Erysiphe in about 5 days and were most luxuriant following inoculations on young mildew colonies. The dish culture method was successfully used for cross inoculations with different isolations of Cicinnobolus on different mildews.

USE OF ENTIRE LEAVES

In a few experiments inoculations with clover mildew were made on entire clover leaves, with the petiole in a nutrient solution in a flask and the lamina in such aerial environment as was desired. Here an accurate comparison of the value of sugar solutions and water as nutrients was not made but it was clearly shown that leaves with their petioles in a weak sugar solution lived longer and supported a more vigorous growth of mildew than leaflets with their petioles in water. However, the optimum concentration of sugar solution under these conditions was only about 2 per cent, much lower than for excised leaflets floating on the solution.

DISCUSSION

There are obviously certain advantages and certain disadvantages in the use of excised leaves in comparison with the use of entire plants in infection experiments. The main disadvantage, already given by Clinton and McCormick (1), is the relatively early death of excised leaves. Another disadvantage is the difficulty of relating results from experiments of this type to results under natural conditions.

Some of the advantages of the method are as follows (the first 4 of these are given by Clinton and McCormick):

1. Economy of space.
2. Economy of host material.
3. Ease and exactness of observation.
4. Reduced danger from contamination. This is especially important with wind-blown fungi, such as powdery mildews, and when physiologic forms are being studied.
5. Uniformity of experimental units. In a species such as red clover genetically uniform experimental material can be secured only by some method of vegetative propagation, and this method with excised leaflets in dish cultures is more expedient than cuttings for many purposes.
6. Ease of control and modification of environmental factors.
7. More luxuriant development and sporulation of parasitic fungi.

SUMMARY

Methods for the culture of a number of parasitic fungi on excised red-clover leaves are described and discussed.

Leaflets maintained in a high degree of vigor by floating on 10 per cent sucrose solution were more susceptible to Uromyces and Erysiphe and less susceptible to Macrosporium and Colletotrichum than leaflets floated on 2 per cent sucrose or on water.

Leaflets removed from the plant in the late afternoon were more vigorous and more susceptible to Uromyces and Erysiphe and less susceptible to Macrosporium and Colletotrichum than leaflets removed from the plant in the early morning.

Young excised leaflets were more vigorous and more susceptible to Erysiphe and less susceptible to Macrosporium and Colletotrichum than old leaflets.

PURDUE UNIVERSITY,
LA FAYETTE, IND.

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SWEET-POTATO RING ROT CAUSED BY PYTHIUM ULTIMUM[1]

R. F. POOLE

(Accepted for publication October 16, 1933)

The writings of Halsted indicate that he observed the ring rot (Fig. 1, A) of sweet potatoes in 1890 (1). In 1894, he attributed its cause to a Fusarium (*Nectria ipomoeae*) (2). Taubenhaus, after studying the disease in 1915, concluded that *Rhizopus nigricans* is the causative organism of both the distinct ring and soft rots (6). Further studies have shown that the ring rot of sweet potatoes is caused by *Pythium ultimum* Trow. It has been shown to cause also rootlet rot and mottle necrosis, which are widely distributed in nature (3, 4). Harter and Weimer described 3 distinct types of decay of sweet potatoes found in the field resulting from *Pythium ultimum* infections. They were classed as band, marble and cheesy types. The marble and cheesy symptoms occurred during high and low soil temperature conditions, respectively (4). The results of studies on the relations of this fungus to ring-rot formation in sweet potatoes and the effect of the time of harvest on the control of ring rot are discussed in this paper.

As has been pointed out by Taubenhaus, ring rot is known to cause heavy loss of sweet potatoes in both bank and house storage and to develop during the first few weeks after the sweet potatoes have been harvested and placed in storage. Mottle necrosis of sweet potatoes was reported as causing losses as high as 40 per cent in fields in New Jersey (4). Ring rot, considered only as a storage disease, caused from 1 to 20 per cent loss in 60 storage houses examined in Delaware, Maryland, and New Jersey (6). These losses, together with those resulting from *Pythium ultimum* decay of rootlets (4) and of new plants immediately after germination, gives the fungus conspicuous rank as a parasite on the sweet potato.

During the harvest period of 1925, *Pythium ultimum* was isolated from Yellow Jersey sweet potatoes in Cumberland County near Vineland, New Jersey. Sweet potatoes generally are harvested 10 to 15 days later in Atlantic and Cumberland Counties than in Gloucester County. The losses from mottle necrosis and ring rot were invariably worse in the former counties. Cold rains fell frequently during this period of delayed harvest. Since 1926 the soft-rot symptom resulting from *Pythium ultimum* infection has been observed each season on Nancy Hall and Yellow Jersey varieties and to a limited extent on Porto Rico strains in both banks and houses in North Carolina. It has been worse in late autumn after the soils accumu-

[1] Published with the approval of the Director of the North Carolina Agricultural Experiment Station as Research Paper No. 67.

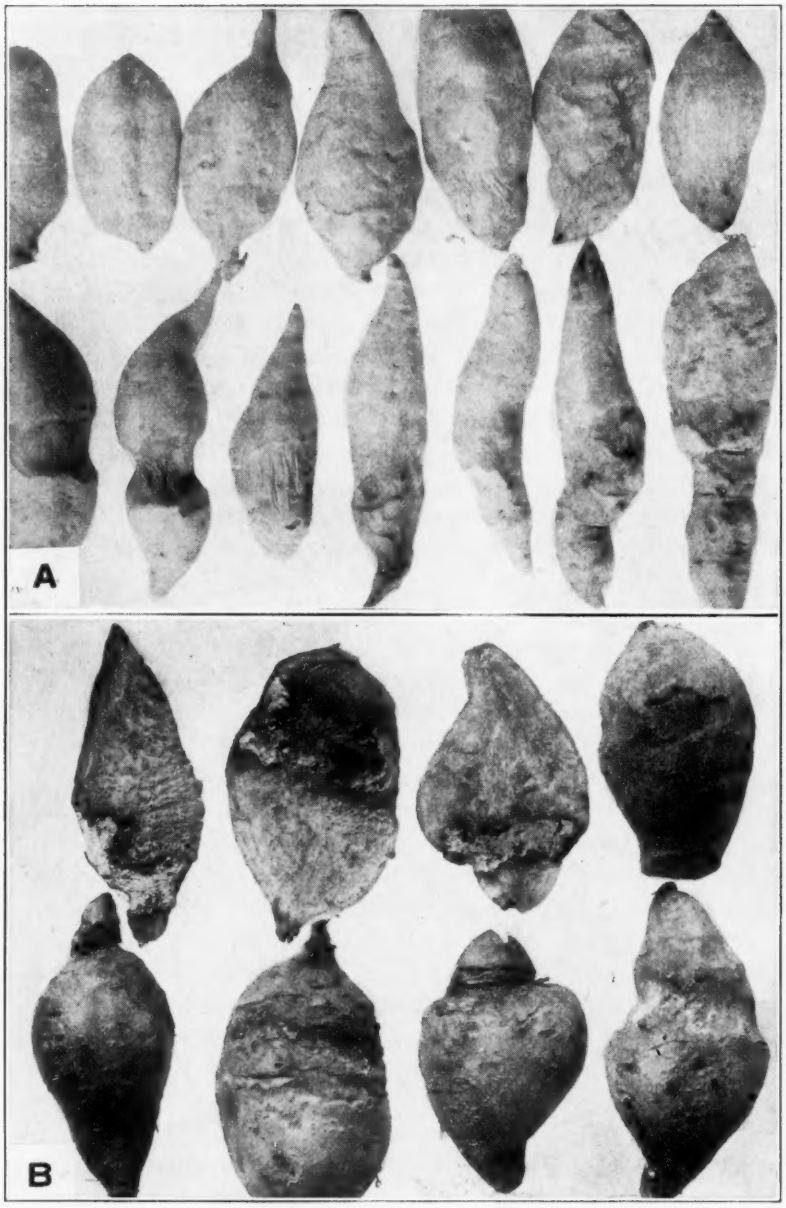

FIG. 1. A. Various symptoms of ring rot on Nancy Hall sweet potatoes collected in storage. B. Sweet potatoes with Trichoderma, Fusaria, and Penicillia growing externally on tissues decayed by *Pythium ultimum*.

lated greater amounts of moisture and the soil temperatures became lower. Isolations were made without difficulty. Pure cultures were readily obtained from platings of tissues from recently decayed sweet potatoes on a medium composed of 250 grams of sweet potato and 12 grams of agar in a liter of water. The fungus was not isolated from the ring rot of sweet potatoes after they had been in storage 4 months. The organism has been grown on many plant decoctions and on synthetic media. Charles Drechsler identified the fungus as *Pythium ultimum* Trow (7). In recent correspondence, he stated that the diseases caused by this fungus had been attributed to *Pythium debaryanum,* even by de Bary.

For studying ring rot in the laboratory, sweet potatoes of a white strain of the Yellow Jersey variety were disinfected by dipping for 5 minutes in 1 : 3000 strength of mercuric chloride. Incisions one-fourth inch deep were made in the sweet potato with a sterile glass tube (Fig. 2, A) or a knife (Fig. 2, C). Inoculations from 1-day-old transfers of pure cultures and infected tissues from recently decayed sweet potatoes were used in making inoculations in the incisions. The inoculated sweet potatoes were incubated at 20° C. in quart jars containing 25 cc. of sterile water. Decay was pronounced 48 hours after inoculation with mycelium from the tubes and in 36 hours when the decayed tissues were used (Fig. 2, A and G). Rings developed rapidly. All tissues of single sweet potatoes became infected in 3 to 7 days, when left in the jars, depending on the size of the sweet potato (Fig. 2, E). The ring symptom was obtained by following the usual procedures used in storing and curing the sweet potato, since, after harvest, the sweet potato is immediately subjected to a temperature of 29 to 30° C. and good ventilation for 2 weeks.

In this study, the inoculated sweet potatoes were removed from the jars immediately after the ring was complete (Fig. 2, A) and dried at 30° C. The diseased tissues dried and shrunk rapidly in 7 days as shown on both sides of the same sweet potato (Fig. 2, B and C). Suppression of further spreading of infection was surprisingly rapid during the drying process. After drying the small sweet potatoes for 7 days, the fungus in the decayed tissues had become inactivated, since attempts to reculture it were unsuccessful.

The definite character of ring rot is easily obtained, and as many as 6 well-marked rings of decayed tissues were obtained from separate inoculations in a single sweet potato (Fig. 2, J). In these studies, 100 per cent infection was readily obtained.

The infected tissues are soft and at first of a grayish color. Extreme softness, such as that resulting from infections of *Rhizopus nigricans,* does not occur. A free liquid is, however, readily squeezed from the tissues of rapidly decaying sweet potatoes. The tissues break firmly, but leave

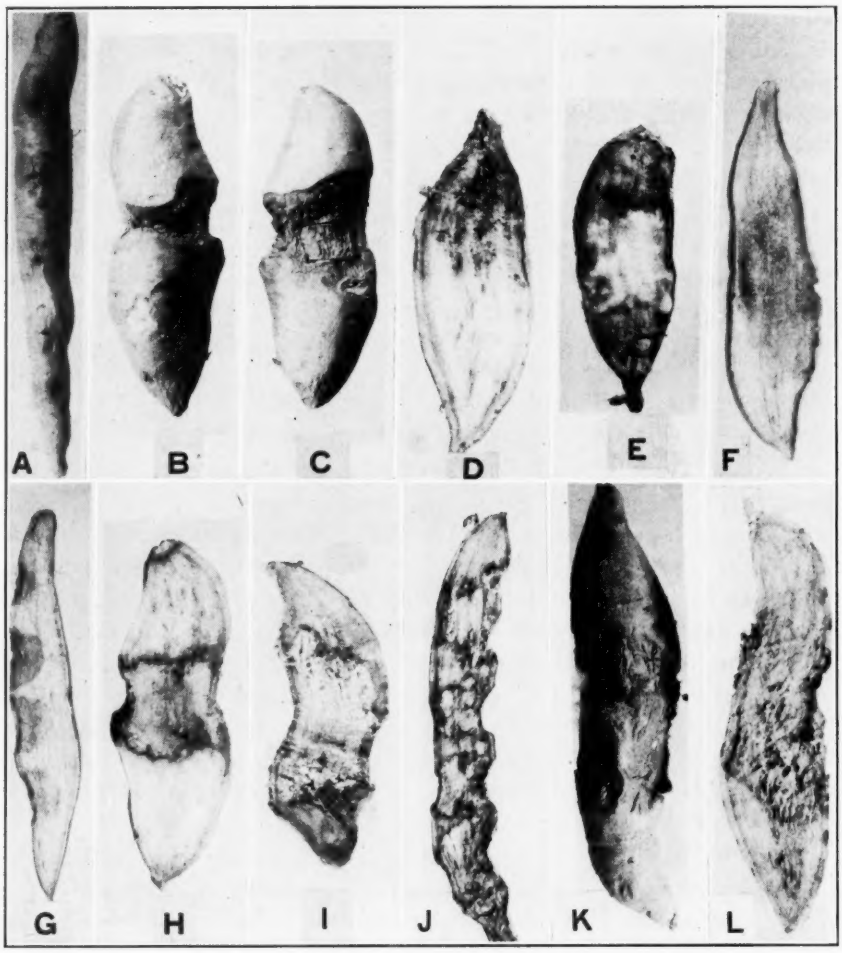

FIG. 2. A. Advancement of decay in sweet potato 36 hours after inoculation with a pure culture of *Pythium ultimum*. B and C. Sweet potato showing ring development on all sides after infected tissues have shrunk from loss of water as a result of pure-culture inoculation. D. End-rot and mottle-necrosis characters resulting from pure-culture inoculations with *P. ultimum*. E. Complete decay and development of white mycelium on cut surface of sweet potato incubated for 7 days in moist atmosphere. F. Black sporangia developing on surface 12 hours after sectioning sweet potato decayed by *Rhizopus nigricans*. G. Section of sweet potato showing advancement of decay 36 hours after inoculation with a pure culture of *P. ultimum*. H. Grayish and soft cheesy characters of diseased tissues and dark oxidized healthy tissues next to infected tissues 24 hours after ring rot was produced. I. Section through ring showing starch that is dry and white in cells after pectin, protein and water have been destroyed by the disease. J. Six rings produced in a single sweet potato by inoculating with *P. ultimum*. K. Type of ring produced by *R. nigricans* showing dark sporangia developing through natural openings and broken tissues of decayed sweet potatoes. L. Stringy and soft tissues of sectioned diseased sweet potato resulting from inoculating with *R. nigricans*.

ragged edges. After the infected sweet potatoes have dried for 24 hours, the decayed parts become moderately soft, somewhat comparable in character to very soft cheese (Fig. 2, H). Later, the dried tissues become loose and crumbly (Fig. 2, I). As a result of the destruction and removal of pectin from the middle lamella, and the removal of protein from the tissues, the decayed and dried parts of the potatoes are nearly white. Water is released to such an extent that the undisturbed, except separated cells full of starch, are prominently displayed (Fig. 2, I). Taubenhaus intimated that the starch was broken down, but his illustrations apparently show heavy starch deposits left in the decayed tissues after decay was stopped (6). Neither *Pythium ultimum* nor *Rhizopus nigricans* displays any definite diastatic action, even up to the time when the fungi cease to be active in the decayed tissues. Slight oxidation occurs in tissues not infected and adjacent to diseased tissues (Fig. 2, I and J). However, the acid reaction that is produced by the fungus in the tissues of the sweet potato, apparently prevents the development of the dark color due to oxidation. Changes in reaction from pH 6.5 in healthy tissues to pH 4.4 in tissues decayed by *Pythium ultimum* and to pH 4.0 in tissues decayed by *Rhizopus nigricans* were observed.

A definite end rot resulted from placing the inoculum of *Pythium ultimum* in the end of the sweet potato and it was seen that the fungus showed a slight tendency to follow the bundles, giving the mottle-necrosis symptom (Fig. 2, D). The mycelium becomes constricted at the cell wall, which it enters by force. There is no evidence of the production of cytase. The combined intercellular and intracellular activities of *Pythium ultimum* would seemingly offer sufficient explanation of the development of the ring character, since infection works from the center of infection uniformly in all directions (Fig. 2, A and G). There is no evidence that *Rhizopus nigricans* enters the cells but it shows greater activities in destroying the middle lamella than does *Pythium ultimum*. This partly accounts for greater decay of the sweet potato as a whole resulting from infection by *Rhizopus nigricans*, as compared to the constricted rings produced by *Pythium ultimum*.

In the field, infection takes place through the stem end and through feed roots on the sweet potatoes (Fig. 3, A). The rootlets are decayed in 3 days. The new plants begin to show decay in 36 hours and continue to decay rapidly (Fig. 3, B). Total decay of young plants was obtained without destroying the mother sweet potato. The mother sweet potato did decay if it became infected later and high moisture conditions were maintained. After infection had become established it continued even under drying conditions until considerable water was removed from the tissues. Temperatures of 29 to 30° C. used during the curing of sweet potatoes in storage prevented

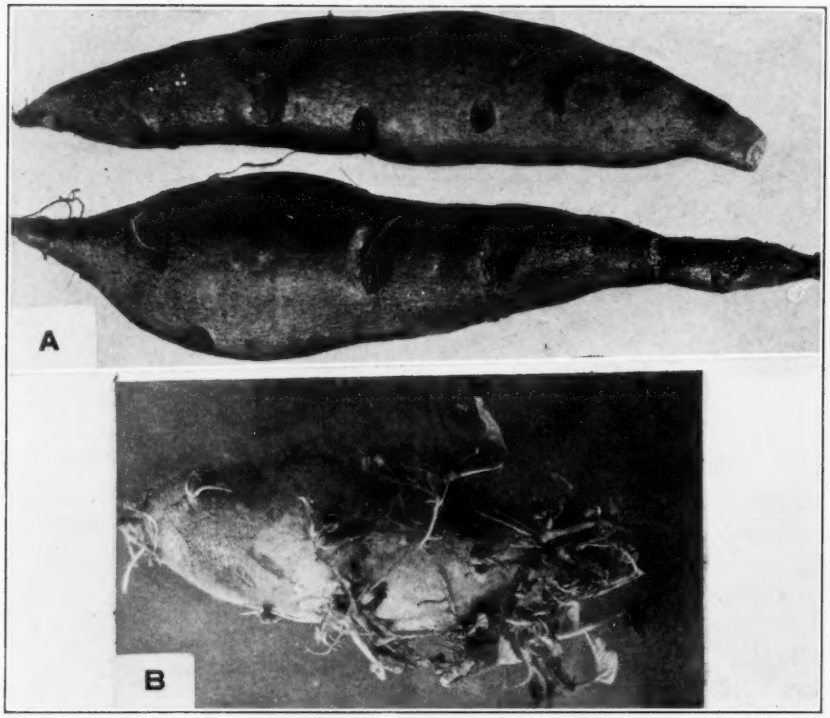

FIG. 3. A. Sweet potatoes showing infections of *Pythium ultimum* at ends and through small roots. B. Rootlets, terminal growth and leaves decayed by *P. ultimum*.

infection, even when large amounts of inoculum were inserted, indicating that infection resulting in ring rot is established in sweet potatoes before they are stored. Infection may, however, frequently occur immediately after the potatoes are stored, since high humidity persists in the various types of storage for 3 or 4 days.

Ring rot was produced readily in Nancy Hall, Triumph, Norton Yam, Red Jersey, Yellow Jersey, and Big Stem Jersey sweet potatoes. Inoculations of Porto Rico, White Yam, and Yellow Yam resulted in decay of a minor character only. String beans, beets, Irish potatoes, turnips, and radishes decayed rapidly. A copious growth of mycelium was produced on all of these plants. Carrots were slightly affected. Artichokes, onions, and parsnips did not decay, even when a heavy inoculum of freshly decayed tissues was inserted in deep punctures.

Results of studying the occurrence of infection in the field and the development of ring rot in storage and in the laboratory clearly indicate that moisture exerts a controlling influence on the growth of the causative fungus. Sweet potatoes harvested and stored by October 15 rarely show

ring rot in storage. Those harvested later, especially following cold rains, result in tremendous losses. Schmidt shows a 5-year average loss of 44.41 per cent in sweet potatoes when harvested and stored after heavy frost in comparison with 5.39 per cent when harvested and stored on October 20, before low temperatures developed in the soil and while the soil moisture was relatively low (5). His results do not indicate the specific causes of the losses, but *Pythium ultimum* was found by the writer to be the cause of sweet-potato decay in most of his tests. Nancy Hall potatoes harvested October 10, 15, 20, 30, November 10, 15, 20, and 30, 1930, showed 0, 0, 3, 8, 15, 30, 60, and 100 per cent pythium decay, respectively. Losses resulting from *Pythium ultimum* infection over a period of 3 years were irregular, since there was considerable variation in the rainfall in these seasons. The disease was produced slowly at 10° C. and more rapidly at temperatures up to 30° C.

Any fungi that produce pectinase and protease, when growing on sweet potatoes, may occasionally produce a rot that could be called ring rot. It would, however, seem unusual to find more than one fungus causing such a specific decay as that of the common ring rot. *Rhizopus nigricans* produces a somewhat disorganized type of ring rot that is definitely unlike the common ring rot (Fig. 2, K). It causes much softness, leaving the sweet potato watery and stringy. The sharply demarked borders, such as are produced by *Pythium ultimum,* were not obtained with *Rhizopus nigricans.* After making 1,000 inoculations with *Rhizopus nigricans,* using vigorous mycelium from pure cultures grown in solution and from freshly decayed tissues, it was shown that this fungus always produces sporangia through the natural openings on the surface (Fig. 2, K). The dark mycelium and skeletonized sporangia also were seen to persist on the decayed potato throughout the storage period. The sweet potatoes affected with the common ring rot rarely show the presence of *Rhizopus nigricans,* but they frequently show such semiparasitic fungi as *Fusarium* sp., *Trichoderma* sp., and *Penicillium* sp. working as saprophytes on the decayed tissues (Fig. 1, C). Sweet potatoes, decayed by *Rhizopus nigricans,* do not break without leaving stringy edges of soft watery tissues that are difficult to cut smoothly (Fig. 2, L). From 12 to 24 hours after sections are made from freshly decayed parts caused by *Rhizopus nigricans,* black sporangia develop in great masses on the cut surface (Fig. 2, F). A white mycelium develops on sections made through sweet potatoes decayed by *Pythium ultimum* (Fig. 2, E). In all respects the symptoms of the common ring rot obtained as a result of pure-culture inoculations with *Pythium ultimum* are exactly like those found in storage and known to develop soon after the potatoes are stored. It is the only time that the disease seems capable of occurring in storage, since the ability of *Pythium ultimum* to perpetuate itself by means of sporangiospores is wanting.

SUMMARY

Pythium ultimum is found to cause ring rot of sweet potatoes. Infection occurs in the field. The greater losses occur after the potatoes are stored, but soft rot in the field is common.

The causal fungus was readily isolated from sweet potatoes in the process of decay. It grows well on a variety of culture media, including sweet-potato decoctions.

Ring rot was produced with the fungus after insertion of the latter into the cortex. Decay was pronounced in 36 to 48 hours after inoculating with an active culture. When the atmosphere was saturated and the temperature maintained at 20° C. in the incubation chamber, 6 well-developed rings were produced on a single potato showing the definite restricted spreading activities of the fungus.

The infection was controlled by harvesting sweet potatoes before extended wet soils favored growth of the fungus in the soil.

Rhizopus nigricans, after many trials, failed to produce the common ring rot.

NORTH CAROLINA STATE COLLEGE OF AGRICULTURE
AND ENGINEERING
RALEIGH, N. C.

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FUNGICIDAL EFFECT ON SCLEROTIUM ROLFSII OF SOME COMPOUNDS IN AQUEOUS SOLUTION AND IN THE GASEOUS STATE[1]

J. OSERKOWSKY[2]

(Accepted for publication September 12, 1933)

THE EFFECT OF FUNGICIDAL VAPORS ON MYCELIUM AND SCLEROTIA

In an attempt to devise a means for the control of *Sclerotium rolfsii*, the fungicidal effect of vapors of various compounds was tested. For the study of the effect of vapors on the mycelium, the following technique was adopted. A small vial was placed upright in a stender dish and melted agar was poured into the dish. After sterilization and cooling, the vial was held in place by the hardened agar. An excess of the volatile fungicide to be tested was placed in the vial and a small block of agar supporting a fresh mycelial growth of the fungus was transferred to the surface of the agar in the stender dish (Fig. 1). The stender dish was placed in an incubator at 29°–

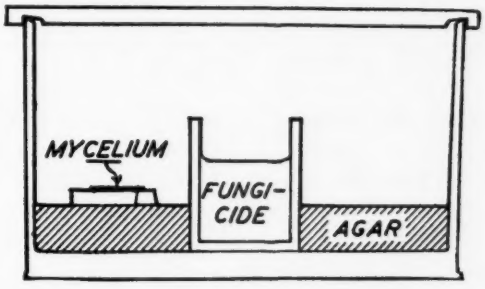

FIG. 1. Schematic drawing of stender dish and arrangement used for testing the fungicidal action of vapors on mycelium of *Sclerotium rolfsii*.

30° C. After 2 to 5 days the mycelial growth in the stender dishes containing the fungicidal vapors was compared with that made by control cultures treated alike in every respect, except that the stender dishes did not contain a fungicide. If no growth was made by the mycelium subjected to the fungicidal vapors, it was transferred to a test-tube containing fresh agar medium free of fungicidal vapors and incubated at 29° to 30° C. for 5 to 22 days; if no growth took place after this period, the fungicidal vapor was considered "lethal."

The vapors of naphthalene and its Cl and Br derivatives were found to

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

[2] It is with appreciation that the writer acknowledges the suggestions and assistance offered him throughout the work by Professors Max W. Gardner and Ralph E. Smith.

completely inhibit mycelial growth. Naphthalene had no lethal effect whatsoever, while α-chlor and α-brom-naphthalene were lethal to a substantial fraction of the number of mycelial inocula used. The vapor tension of naphthalene at 30° C. is 0.09 mm. Hg, that of α-monochlor and α-monobromnaphthalene is approximately 0.03 mm. It is to be inferred from this that the substitution of either Cl or Br for an H atom in the α position in the naphthalene molecule resulted in an enhanced toxicity to the mycelium of *Sclerotium rolfsii.*

Benzene and all its derivatives listed in table 1 were shown to be lethal to the mycelium. The vapor pressure of these compounds at 29° to 30° C. is considerably above that of naphthalene and its halogen derivatives referred to above. It is doubtful whether the benzene derivatives mentioned in table 1 would prove lethal or even growth-inhibitive at vapor pressures corresponding to those of naphthalene and its two halogen derivatives.

TABLE 1.—*The effect of saturated vapors of various substances at 29° to 30° C. on mycelial growth of Sclerotium rolfsii*

Vapors tested	Days of exposure	Number of cultures exposed	Number of cultures showing growth at the end of exposure	Number of cultures showing growth after transfer to fresh agar media
Paraffin oil, yellow	5	13	13	.....
Ethyl laurate	3	6	6	.....
Lead naphthanate	3	17	17	.....
Arsenic trichloride	3	15	0	.....
Trioxymethylene	3	46	0	0
Naphthalene	3	22	0	22
α-monochlornaphthalene	3	35	0	26
α-monobromnaphthalene	3	40	0	21
Benzene	2	26	0	0
Toluene	4	27	0	0
Xylene	3	24	0	0
o-dichlorobenzene	3	50	0	2
m-dichlorobenzene	2	15	0	0
m-dichlorobenzene	3	19	0	0
p-dichlorobenzene	3	20	0	0
Nitrobenzene	3	22	0	0

The inhibitory and perhaps lethal effect of $AsCl_3$ vapors may be due either to $AsCl_3$ as such or to HCl and H_3AsO_3, which are readily formed in the presence of moisture.

When the effect of vapors on sclerotia was investigated the following procedure was adopted. The sclerotia were placed on the bottom of a weigh-

ing glass equipped with a ground-glass cover and containing a small vial of the substance to be tested. The whole was incubated for 3 days at 25° C. The sclerotia were then removed from the weighing glass, planted on agar media, and incubated at 29° to 30° C. The results are recorded in table 2. The data contained in this table were attained with dry sclerotia, the effect of the vapors on moist sclerotia may be different.

Since the containers used were small and closed during exposure of the fungus to the vapors of the fungicides it is safe to assume that the concen-

TABLE 2.—*Effect of exposure of sclerotia of Sclerotium rolfsii to vapors of various compounds for three days at 25° C.*

Compound	Number sclerotia tested	Percentage of sclerotia germinated	Compound	Number of sclerotia tested	Percentage germination of sclerotia
Benzene	85	0	Aniline	82	94
Toluene	61	0			
Ethylbenzene	38	0			
Xylene	69	0	Mercury-di-p-tolyl	238	96
n-propylbenzene	61	0			
n-butylbenzene	49	78			
Tertiary amylbenzene	59	100	Naphthalene	62	98
			α-monobromonaphthalene	51	92
Chlorobenzene	46	0			
o-dichlorobenzene	69	0			
m-dichlorobenzene	113	0	Chloroform	59	0
p-dichlorobenzene	47	100	Carbon tetrachloride	80	0
1, 3, 5, Trichlorobenzene	63	100	Trichlorethylene	97	96
1, 2, 4, Trichlorobenzene	56	0	S-Tetrachlorethane	78	96
1, 2, 4, 5, Tetrachlorobenzene	53	93	Pentachlorethane (Tech)	102	100
Hexachlorobenzene	68	100	Chloropicrin	128	100
o-chlorotoluene	40	100			
m-chlorotoluene	110	94			
p-chlorotoluene	74	99			
			Bromoform	90	18
			Bromopicrin	90	0
Bromobenzene	88	100			
o-bromotoluene	118	89			
p-bromotoluene	54	100	Iodine	47	0
			Iodoform	79	100
Nitrobenzene	32	0			
m-dinitrobenzene	84	100	"Oronite crystaloil"	83	100
p-dinitrobenzene	64	100	"Chlorox"	91	100
1, 3, 5, Trinitrobenzene	87	100	Carbon bisulphide	25	0
m-nitrotoluene	95	100	Trioxymethylene	37	0
o-nitrotoluene	115	96			
			Checks	956	97
2–5 dichloronitrobenzene	356	94			
3–4 dichloronitrobenzene	327	92			

tration of the vapors in the containers was close to the vapor tension of the substances investigated at 25° and 30° C., respectively. For most substances used in this study no data are available regarding their vapor tension at these temperatures; hence, no general basis of comparison of the relative toxicity of these compounds is available. In a few instances, however, where the vapor tension at 25° C. could be obtained, either directly from tables or by graphical extrapolation and intrapolation, it was possible to compare toxicity and chemical structure (Table 3). Thus it was found

TABLE 3.—*Chemical composition, vapor tension and effect of vapor on sclerotia of Sclerotium rolfsii*

Compound	Formula	Vapor tension at 25° C. mm. Hg.	Effect on sclerotia
Nitrobenzene	$C_6H_5NO_2$	0.41	lethal
Aniline	$C_6H_5NH_2$	0.59	not lethal
Bromobenzene	C_6H_5Br	5.05	not lethal
p-dichlorobenzene	$p\text{-}C_6H_4Cl_2$	0.86	not lethal
Bromopicrin	CBr_3NO_2	<15	lethal
Chloropicrin	CCl_3NO_2	18.3	not lethal

that the substitution of an NO_2 group in the benzene ring resulted in a greater toxicity than the substitution of one NH_2 or one Br, or 2Cl atoms in the para position. Similarly, the substitution of Br for Cl in chloropicrin resulted in enhanced toxicity. It was not possible to arrive at the vapor tension of bromopicrin at 25° C. At 85° to 86° C. its vapor tension is 15 mm. It must be, therefore, substantially lower at 25° C. Iodine, with a vapor tension of 0.3 mm. at 25° C., was lethal to sclerotia of *Sclerotium rolfsii*, and experimental evidence indicates that it is lethal even at a substantially lower vapor pressure.

THE EFFECT OF AQUEOUS SOLUTIONS OF VARIOUS SUBSTANCES ON SCLEROTIA

Sclerotia were put in small cheesecloth bags and immersed in solutions of various substances for several days at 25° to 26° C.; then each bag was passed successively through 5 flasks, containing sterilized distilled water, to remove as much as possible of the fungicidal substance. The sclerotia were then planted on agar media and incubated at 29° to 30° C. In a few instances the sclerotia were dipped in 0.1 per cent $HgCl_2$ for 80 to 135 seconds after removal from the fungicidal solution and previous to passing through the sterilized-water flasks for the purpose of surface sterilization. Although the $HgCl_2$ treatment may have decreased somewhat the number of sclerotia germinating, it did not materially affect the results. Some of the solutions seemed to cause a decrease in percentage of germinating sclerotia, but in view of the wide variation in the percentage of germination

in duplicate treatments, it is doubtful whether any significance can be attached to these low germination values. It was deemed advisable, therefore, to omit the percentage of germination from the following data and to divide the substances into two groups, namely, those that completely inhibited germination, and those that did not inhibit it completely. To the first group belong Merthiolate (33[1], 1), Hexylresorcinol[2] (1000, 1) and o-chlorophenol (1000, 5). The following substances are included in the second group: p-chlorophenol (500, 3), o-chlorophenol (1000, 1), 2-chloro-2hydroxytoluene (200, 3), picric acid (500, 4), sodium salicylate (350, 3), paraldehyde (1000, 3) saturated solution of p-nitrochloro-benzene (3 days), potassium bromide (300, 3), $Na_2S \cdot 9H_2O$ adjusted to pH 6.4 (1750, 4), sodium chlorate (1000, 3), sodium sulphite (500, 3), mercurochrome (195, 3), lead nitrate (185, 1) and $CuSO_4 \cdot 5H_2O$ (200, 3).

With few exceptions the concentrations tried corresponded to what were considered to be the maximum concentrations compatible with practical use. It is unfortunate that precisely the three treatments that completely inhibited germination of sclerotia, *i.e.*, killed the sclerotia, cannot be used for practical purposes because of their prohibitive cost, even if they should prove as effective in soil as they are *in vitro*.

SUMMARY

Mycelium of *Sclerotium rolfsii* was not killed, but its growth was inhibited during 2 to 4 days' exposure to saturated vapors at 29° to 30° C. of naphthalene, while the vapors of α-monochlor-napthalene and α-mono-brom-naphthalene inhibited growth of mycelium and were lethal to some of the cultures.

The mycelium was killed by the saturated vapors of trioxy-methylene, benzene, toluene, xylene, nitrobenzene and ortho, meta and para dichlorobenzene.

Sclerotia were killed by three days' exposure at 25° to 26° C. to the saturated vapors of benzene, toluene, xylene, ethylbenzene, n-propylbenzene, chlorobenzene, ortho and meta dichlorobenzene, 1, 2, 4 trichlorobenzene, nitrobenzene, chloroform, carbon tetrachloride, bromopicrin, iodine, carbon bisulphide, and trioxymethylene.

Sclerotia were killed also after immersion for one day in 1:33 dilution of Merthiolate, and in 0.1 per cent solution of hexylresorcinol, and for 5 days in 0.1 per cent of o-chlorophenol.

Substitution of an NO_2 radical in the benzene ring resulted in a greater toxicity than the substitution of either NH_2, Br, or 2 Cl atoms in the para position. Substitution of Br for Cl in chloropicrin enhanced the toxicity.

[1] 1:33 dilution of the commercial product "Merthiolate."

[2] In the following list the first number in the parenthesis refers to the concentration of the substance in parts per million by weight, the second number indicates the number of days the sclerotia were immersed in the solution.

CUCURBIT MOSAIC TRANSMITTED BY MUSKMELON SEED[1]

JAMES B. KENDRICK[2]

(Accepted for publication October 3, 1933)

In November, 1932, a single case of mosaic occurred in 170 seedling Persian melons growing in sterile soil in the greenhouse, at Davis, California. The spontaneous occurrence of a single mosaic plant in a large number of seedlings strongly suggested the seed as the source of the disease.

According to Doolittle and others, the cultivated species of cucurbits rarely, if ever, transmit mosaic through the seed. Doolittle and Walker[3] report one case of apparent seedling infection in approximately 22,000 cucumber plants and negative results in less extensive trials with muskmelon, squash, and pumpkin seed harvested from mosaic plants. It has been shown, however, that seed of the wild cucumber, *Micrampelis lobata*, is capable of transmitting mosaic.[4]

In order to determine whether or not the mosaic virus might be transmitted through muskmelon seed, 23 ¼-lb. packets of 12 commercial varieties of muskmelon seed were secured from 3 different seedsmen. On March 14, 1933, seed from 14 packets, representing 11 varieties, were used to plant flats of steam-sterilized soil that were placed on a greenhouse bench. Repeated fumigation to keep out insects and the absence of cucurbit mosaic in the greenhouse precluded the possibility of introduction of the virus from sources other than the seed.

Two weeks after planting, the young plants were developing the first foliage leaves, and evidence of mosaic was present on such leaves of three plants in a flat of Honey Dew melons grown from seed packet number 1 (Fig. 1). On April 8 all plants were examined critically for mosaic symptoms, and one additional mosaic plant was found in a flat of plants from Persian seed packet number 1. The totals are presented in table 1.

As a further check on the above results, on April 11, seed from 16 packets representing 7 varieties, was planted in the same flats of soil without resterilization. In 16 days, the plants were developing their second foliage leaves and were carefully examined for evidence of mosaic. It was found that 15 of the 268 plants from Honey Dew, seed packet number 1, and one

[1] Contribution from the Division of Plant Pathology, Branch of the College of Agriculture, Davis, California.

[2] The writer wishes to express his appreciation to Professors Ralph E. Smith and Max W. Gardner for helpful advice and criticism.

[3] Doolittle, S. P., and M. N. Walker. Further studies on the overwintering and dissemination of cucurbit mosaic. Jour. Agr. Res. 31: 1–58. 1925.

[4] Doolittle, S. P., and W. W. Gilbert. Seed transmission of cucurbit mosaic by the wild cucumber. Phytopath. 9: 326–327. 1919.

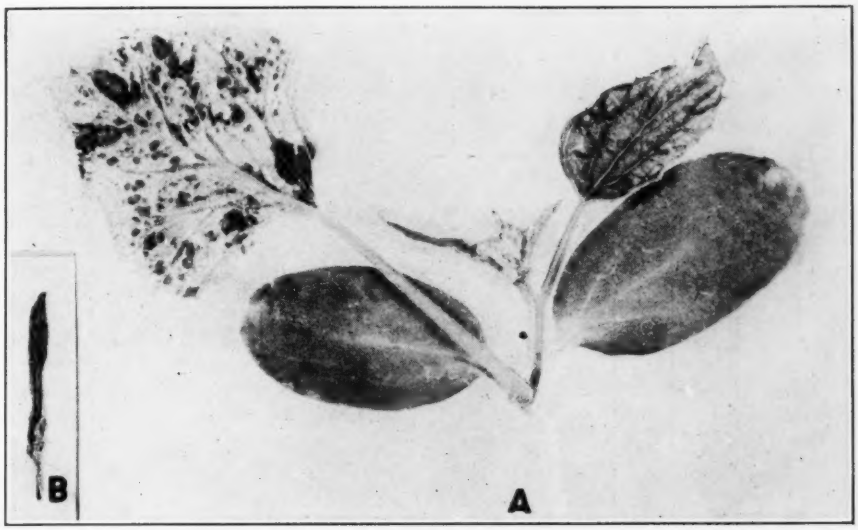

FIG. 1. A. Mosaic symptoms on a seedling Honey Dew muskmelon plant grown from commercial seed. Note the irregular dark green raised areas on the lighter green background of the first foliage leaf and the large blister-like areas on the somewhat elongated second foliage leaf. The third and fourth foliage leaves show extreme elongation and a slight upward curling of the edges. B. The third foliage leaf detached from the plant.

plant from Honey Dew, seed packet number 4, showed mosaic, while no mosaic was found in plants from the seed of the other 14 packets (Table 1).

In order to preclude all possibility of an outside source of the seedling mosaic, the flats of soil were again steam-sterilized, planted with seed from the 3 packets that had previously yielded evidence of seed transmission, and immediately covered with insect-proof cages. The cages were removed 20 days after planting and the plants examined for mosaic symptoms. There were 7 cases of typical mosaic in 513 plants from Honey Dew, seed packet number 1, while 332 plants from Honey Dew, seed packet number 4, and 399 plants from Persian, seed packet number 1, were all healthy (Table 1).

Table 1 shows that in the 1,171 plants grown from Honey Dew, seed packet number 1, 25, or 2.13 per cent, were mosaic. In the 656 plants from Honey Dew, seed packet number 4, 1, or 0.15 per cent, was mosaic and in the 1,061 plants from Persian, seed packet number 1, 1, or 0.094 per cent, was mosaic. No mosaic occurred in the plants grown from the other 20 seed packets. Out of the total of 11,519 plants grown from the 23 packets of muskmelon seed, 27, or 0.23 per cent, were mosaic.

The mosaic was successfully transferred by artificial means from the mosaic Honey Dew seedlings to the Casaba, Hale's Best, Honey Ball, Honey

TABLE 1.—*Summary of greenhouse trials with 23 packets, representing 12 varieties, of commercial muskmelon seed under controlled conditions for seed transmission of cucurbit mosaic*

Variety and packet[a]	Trial, March 14 to April 8		Trial, April 11 to April 27		Trial, May 9 to May 29		Totals		
	Number plants	Number mosaic	Number plants	Number mosaic	Number plants	Number mosaic	Number plants	Number mosaic	Per cent mosaic
Burrell Gem	371	0					371	0	
Casaba (1)			452	0			452	0	
" (2)	256	0	542	0			798	0	
Hale's Best (1)	633	0					633	0	
" " (2)	205	0					205	0	
" " (3)			388	0			388	0	
" " (4)			493	0			493	0	
Honey Ball (1)			351	0			351	0	
" " (2)			253	0			253	0	
" " (3)			197	0			197	0	
Honey Dew (1)	390	3	268	15	513	7	1171	25	2.13
" " (2)	271	0	413	0			684	0	
" " (3)			264	0			264	0	
" " (4)			324	1	332	0	656	1	0.15
Honey Rock	394	0	420	0			814	0	
Lake Champlain	334	0					334	0	
Osage	335	0					335	0	
Pearl Pink Meat	421	0					421	0	
Perfecto Improved	402	0					402	0	
Persian (1)	261	1	401	0	399	0	1061	1	0.094
" (2)			281	0			281	0	
" (3)			362	0			362	0	
Tip Top	352	0	241	0			593	0	

[a] Where more than one packet of a variety was tested the packets were numbered to designate the source of the seed.

Dew, and Tip Top varieties of muskmelons and to the Early White Bush Scallop, Giant Summer Crookneck, and Italian varieties of squash. In each case 100 per cent infection was secured, and symptoms comparable in every way to the disease on the seedling Honey Dew plants appeared in 10 to 12 days.

SUMMARY

The above tests indicate that cucurbit mosaic can be transmitted with muskmelon seed. In controlled experiments with 23 packets, representing 12 varieties of commercial muskmelon seed, a small percentage of successful seed transmission was secured from 3 of the 23 packets.

PHYTOPATHOLOGICAL NOTES

Reaction of Alfalfa Varieties to Stem Blight.[1]—Stem blight[2] is an important disease of alfalfa in Utah; it is also a factor that will function prominently in determining the relative value of any alfalfa variety to be introduced into the State or possibly into any part of the intermountain region of the United States. During the crop season of 1933 the writer had an excellent opportunity to study the reaction of a number of alfalfa varieties to alfalfa stem blight under conditions that appear to add special significance to the information obtained. The important relation of this information to the possible value and general adaptability of recommended wilt-resistant varieties of alfalfa seems to the writer to justify an early statement, even though this information presented is based upon only a year's observations.

Source of Data and Method of Collection

The varieties of alfalfa listed in figure 1 were planted in 1932 in plots 18×21.5 feet and so randomized as to give effective distribution of related varieties over the entire field.[3] A number of varieties were replicated 3 times, others twice, and a few were grown in single plots. These plantings were made primarily for the purpose of testing these varieties to determine their relation to bacterial wilt.

The spring of 1933 was especially cold and wet. Low temperatures prevailed throughout the greater part of May, with killing frosts on May 9 and 11. This resulted in severe frost injury to nearly all varieties, which at this time ranged in height from 4 to 8 inches. Following this injury, alfalfa stem blight developed throughout the entire field, resulting in extremely severe damage, particularly to certain varieties. The disease was so severe as to reduce yields in these varieties in the first crop as much as 40 to 50 per cent. Final observations on the degree of severity of the disease in the various varieties were made on June 16 and 20.

In making observations a wire hoop, 18 inches in diameter, was placed at random in various plots. The stems thus encircled were cut close to the ground, collected, and classified according to the amount of alfalfa stem

[1] Contribution from Department of Botany and Plant Pathology, Utah Agricultural Experiment Station. Publication authorized by the Director, March 13, 1934.

[2] The specific etiology of the disease here designated as stem blight was not determined. While Sackett's work on etiology has been generally accepted, it is important to note that the author has isolated a Phoma species with considerable uniformity. This fact is especially significant in view of the recent work of Johnson and Valleau in Kentucky on black stem of alfalfa and other legumes.

[3] Data were collected from wilt-resistant varieties planted cooperatively by R. J. Evans, Agronomist, and the writer.

blight with which they were affected. Three loops, collected from each plot, had an average of 160 stems per loop, or approximately 450 to 480 stems per plot. The total number of stems for each variety used was 1, 2, or 3 times the average of 450 per plot, dependent upon the number of replications of the variety.

Derivation of a Coefficient of Varietal Susceptibility[4]

All diseased stems collected were segregated into 6 classes, dependent upon the degree of severity exhibited: (1) Very light, (2) light, (3) average, (4) severe, (5) very severe, and (6) dead. These classes were then assigned consecutive numbers from 1 to 6, inclusive, each consecutive number corresponding to the relative increased degree of severity: No 1 indicated least and No. 6 the greatest severity.

The number of stems in each diseased class was multiplied by the index number for the class; the sum total of numbers thus derived was divided by the total of diseased and disease-free stems collected from each plant. This figure, which represented an average of figures derived from the various replications, was then accepted as the coefficient of susceptibility for each variety of alfalfa studied. Coefficients for the various alfalfas are shown in figure 1.

TABLE 1.—*The comparative severity of alfalfa stem blight in typical plots of Ladak and Turkestan alfalfa in Salt Lake County, Utah*

Degree of severity	Variety	
	Turkestan	Ladak
Class	*No. stems*	*No. stems*
Free	16	279
Very light	116	21
Light	120	3
Medium	74	0
Severe	14	0
Very severe	1	0
Dead	2	0

The severity of stem blight in so large a number of varieties as were tested suggests the desirability of combining both stem blight and wilt resistance in all alfalfa varieties to be grown under Utah conditions. This will be especially true for varieties to be grown in the higher valleys in the State, as well as throughout the intermountain section as a whole.

[4] The word "susceptibility" is used here primarily in the sense of degree of severity of the disease exhibited in the different varieties. No specific resistance in those varieties that remained free or relatively free from the disease is definitely implied.

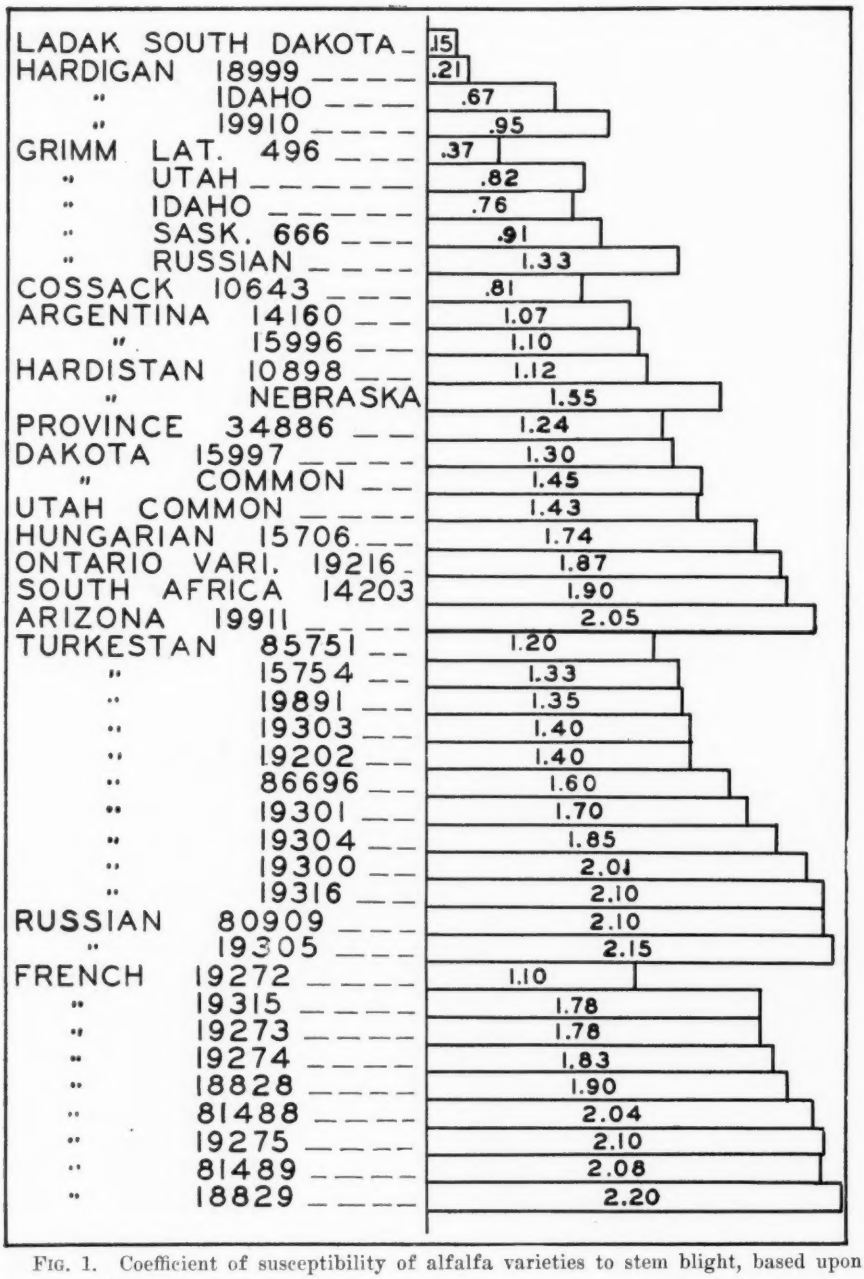

FIG. 1. Coefficient of susceptibility of alfalfa varieties to stem blight, based upon one year's observations only.

The significance of one year's (1933) observations as to the degree of true resistance exhibited in various alfalfas to stem blight, as well as the nature of resistance involved remains to be determined by further detailed observations and by extensive artificial inoculation studies. B. L. RICHARDS, Utah Agricultural Experiment Station, Logan, Utah.

Black Spot of Germinating Pea Seed.[1]—During the past several years a peculiar black spotting of germinating pea and bean seed has been noted in the seed-testing laboratory. The trouble has become more noticeable each year until, in 1933, blackened seeds were found in all of several hundred samples of peas, beans, and Lima beans representing the commercially important varieties. Lots from every principal seed-growing region, as well as from local acreages, were spotted abundantly.

The spots are dull olivaceous to sooty black, and appear most strikingly at the termination of the usual 6-day germination period. They are particularly evident on white peas and beans, but can be detected readily on green pea seed, less so on red, and only with difficulty on brown and black seeds. Samples germinated at 25° C. usually contain more spotted seeds than do samples germinated at 20° or 30° C. In some cases a single spot may seem to envelope the entire surface of a seed, and more frequently, as many as 50 to 100 minute spots may be present; but most of the contaminated seeds are partially blackened by from 3 to 10 spots, varying from 1 to 5 mm. in diameter (Fig. 1, A). The spots appear to be confined to

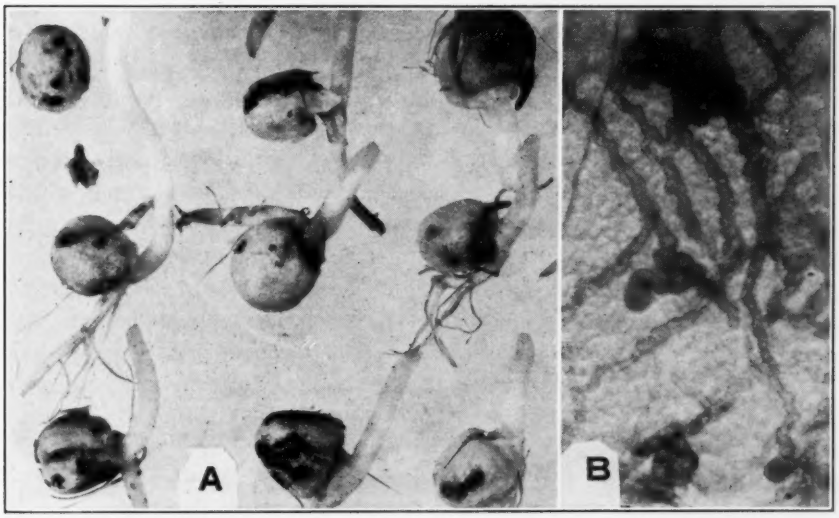

FIG. 1. *Dematium pullulans* (?) on peas. A. Mycelial masses on germinating seeds. B. Photomicrograph of hyphae on a seed coat. ×220.

[1] Approved for publication by the Director of New York State Agricultural Experiment Station as Journal Paper No. 34.

the seed coats, since smearing infected tissues over the cotyledons has never resulted in the formation of the typical black areas.

The blackened areas are superficial masses of fungus hyphae. The mycelium consists of long many-septate hyphae that in most cases consist entirely of black or dark-brown cells but in some instances of hyaline and alternate hyaline and black cells. The hyphae vary greatly in diameter, 4 μ to 16 μ, and the diameter of a single hyphae is rarely uniform over any considerable length. The septa may occur so close together that the width of a cell exceeds the length, although (Fig. 1, B) the length is usually 1½ to 2 times the width. In fully developed hyphae constrictions at the septa create the impression of long strands of catenulate spores, not unlike chlamydospores of certain species of Fusarium or of so-called spores of Torula species. Branching occurs very irregularly and appears to be due to budded outgrowths from the lateral walls of the cells. Short-ovate to cylindric, hyaline spores, 3.5–6.3 μ × 6.5–12.5 μ, develop from the walls of the hyphae on short sterigmata. They are present infrequently on the seed coats but form rapidly when infected tissue is placed in water.

Since no perfect stage has been found, the fungus is regarded as a member of the family Dematiaceae. It has not been assigned definitely to any species but its appearance and habits of growth suggest the fungus known as *Dematium pullulans* de By. et Löw.[2]

The extremely common occurrence of the fungus on pea and bean samples indicates that it is distributed easily in the field or at the growing stations or else it is spread from sample to sample in the germination room. The latter thought was considered highly probable, since each sample in a large shipment of peas is placed on wet paper toweling with a counting board. The board becomes moist through contact with the damp towels so that any adhering spores might easily become distributed to other samples. It has been noticed that samples placed on the towels by hand always contain fewer spotted seeds than do those handled by the counter method. Comparative tests employing the 2 methods have demonstrated definitely that the counter may distribute the fungus to other samples, but since most samples are infested when they arrive at the laboratory, this form of contamination is of negligible importance. Green peas collected direct from the pods with sterilized forceps failed to show any spots, thus indicating that infestation must occur after the seeds are harvested and threshed. Further opportunity for contamination occurs in the grading, bagging, and elevation operations. Ample proof of this possibility was evidenced when green peas, taken direct from clean pods and then mixed with dried spotted tissues, developed the typical black areas.

[2] Drs. C. L. Shear, F. A. Wolf, F. L. Stevens and A. E. Jenkins kindly examined cultures for which thanks are gratefully given.

Although all indications negated an active pathogenicity, experiments were outlined to determine definitely this point. Pea and bean plants of 49 varieties were inoculated at various ages and by different means. Water and decoction suspensions, agar cultures, and infected tissues were employed. Spraying or rubbing the fungus on the foliage, pods, or blossoms, or puncturing it into the stems or pods gave negative results. Attempts to develop the fungus on seedlings in the germinators also were unsuccessful.

The writer would welcome any information from pathologists or seed-laboratory workers who may have observed this black spotting on germinating peas.—WILLARD CROSIER, New York State Agricultural Experiment Station, Geneva, New York.

A Modification in Incubator Construction.—An incubator of large capacity being required and the necessary funds for its purchase unavailable, the incubator herein described was designed and built at a cost of about one-third that of standard commercial types. It has a volume of 18.5 cubic feet and a shelf space of 4030 square inches. The interior is 42 inches high by 30 inches wide by 26 inches deep, and is provided with 5 heavy wire shelves. The construction of the walls is shown in Figure 1, A. The heat insulation of each wall consists of $\frac{1}{8}$ inch pressboard, $\frac{1}{4}$ inch air space, $\frac{1}{2}$ inch insulation board, $\frac{1}{4}$ inch air space and $\frac{1}{2}$ inch insulation board from inside to outside in the order given.

Air circulation is provided through the arrangement shown in figure 1, B. By means of the false top and side walls, a 1-inch space is left between the incubator interior and the external walls through which warm air is forced by a blower. This warm air first passes through the top-wall air space and then down the side-wall air spaces to emerge through 1-inch slots at the bottom of the compartment. Passing up through the incubator interior, the air enters the control box on top where it is warmed, if necessary, before again entering the blower (Sirocco 00). This arrangement would appear to have several advantages over conventional arrangements. The air is circulated and all of it passes over the thermostat instead of attempting to maintain temperature uniformity through air circulation by temperature difference in the incubator interior. Temperature differences in the compartment are avoided also by placing the heaters outside, *i.e.*, in the control box. The warm air, before entering the interior, warms it by conduction from the top and side air spaces. Lastly, heat can be lost from the interior only through 3 (back, front, and bottom) of its sides.

The interior is free of all control equipment and electrical parts, excepting a 10-watt lamp to furnish light and the thermostat in the air outlet. These are located on top of the incubator. The heater (75-, 150-, 300-watt) is in the interior of the control box at the blower inlet while the switches, relay, and indicator lamp are mounted on the front of the control box.

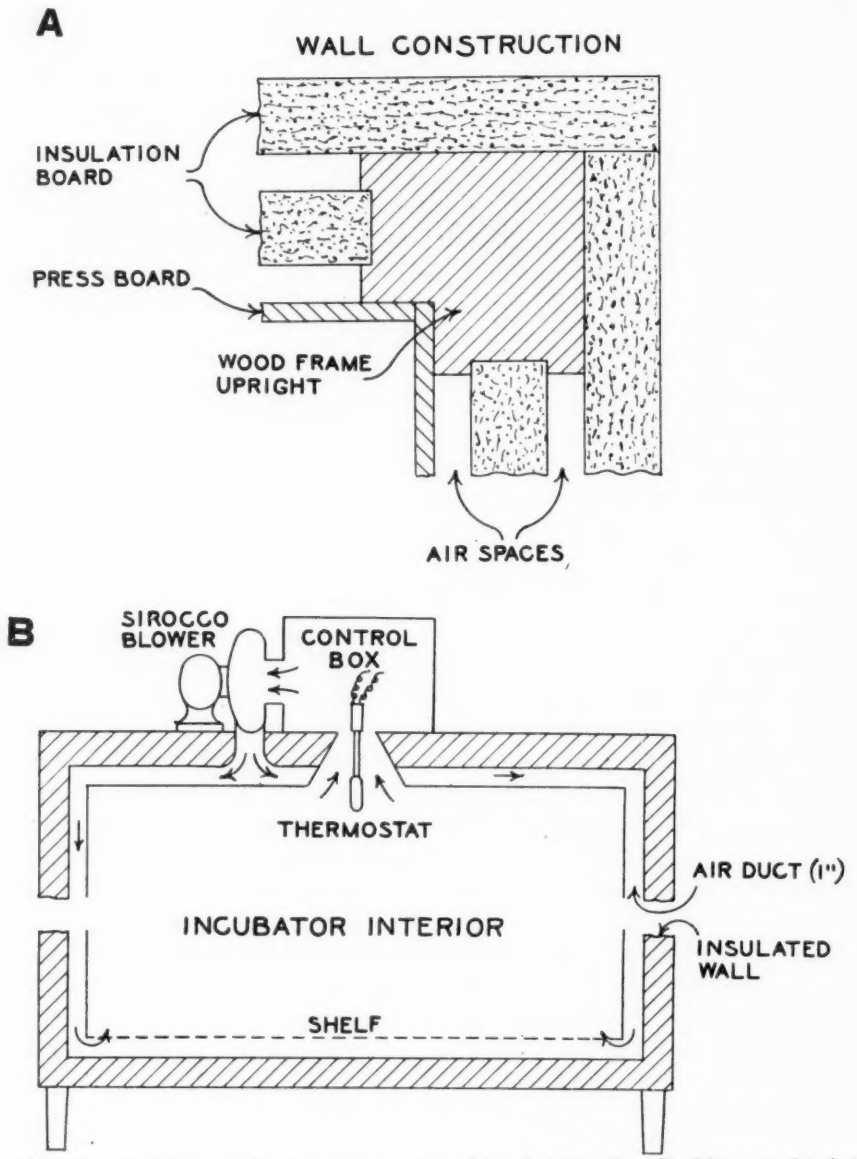

FIG. 1. A. Diagram showing construction of incubator walls. B. Diagram showing interior of incubator.

Temperature control in the compartment, the incubator being in a room at ordinary temperature, and a glass-mercury thermostat sensitive to 0.1° C. being used, is to $\pm$ 0.15° C. at a temperature of 35° C. Enough heat is furnished by the 150-watt heater (and by the blower) to maintain this tem-

perature. No difference in temperature could be detected with a thermometer in different parts of the incubator. Air circulation, or draft in the compartment, on the other hand, is barely perceptible to the hand, and, being directed from below upward, it cannot lift even fine powders from their containers.

Illustrative of the precision obtainable through use of this incubator the following may be cited. The dry-weights in milligrams of two pairs of duplicate cultures of *Aspergillus niger*, kept in different parts of the incubator, were 609.1 $\pm$ 3.5 per cent for the one pair, and 630.8 $\pm$ 5.3 per cent for the other pair. The average for the two pairs was 645.0 $\pm$ 2.2 per cent. In another case the averages of the two pairs were 476.7 $\pm$ 1.5 per cent and 467.8 $\pm$ 0.3 per cent., the average for the two pairs being 472.3 $\pm$ 1.0 per cent.—ROBERT A. STEINBERG, Bureau of Plant Industry, Washington, D. C.

Stereum gausapatum, Cause of Heart Rot of Oaks.—Numerous fungi cause decay in living hardwood trees of the eastern United States and usually several species are responsible for similar types of decay. For example, *Polyporus sulphureus, P. spraguei,* and *Daedalea quercina* all produce brown carbonizing rots, and *Fomes everhartii, F. igniarius,* and *F. rimosus* cause soft, stringy decays. The "piped" or white-lined type of decay also is produced by several different fungi so that it is difficult to determine the causal organism from decayed wood alone. On account of this complication the Division of Forest Pathology is attempting to identify culturally the causal organism of each decay collected, by comparison with a reference collection of cultures made from sporophores of fungi known to induce such decay.

In the spring of 1933, a large percentage of the cultures obtained from decays of oak was of a fungus that differed from any of the well-known decay fungi. In initial stages of decay this fungus forms white lines through the sound wood causing a mottled appearance. These white lines or channels usually follow the spring wood vertically, but they branch frequently and sometimes penetrate through the annual growth rings. Later, much of the spring wood becomes decayed and, in the final stages, all the wood becomes light colored and brittle.

A Stereum fruiting body, later determined as *S. gausapatum* Fr. by L. O. Overholts, was associated on one tree with the decay that gave the above-mentioned fungus. The fungus isolated from the decay was identical with spore cultures of *S. gauspatum* made by Lawrence L. Sluzalis in 1932. The writer has since obtained 5 additional spore cultures of *S. gausapatum* and all of these seem to be the same as the decay fungus. Several of the cultures isolated from decayed wood have formed abnormal Stereum-like fruiting bodies in culture, but no spores were produced on them.

On examining a large collection of unidentified decay cultures that have accumulated since 1928, it was found that many were of this same fungus. These, together with those obtained by the writer, indicate that *Stereum gausapatum* is common as a heart-rotting organism in living oak trees. It has been obtained in culture from 6 *Quercus prinus*, 13 *Q. alba*, 5 *Q. coccinea*, 7 *Q. velutina*, 4 *Q. rubra*, and 4 *Quercus* sp. These were collected in North Carolina, West Virginia, Virginia, Pennsylvania, New Jersey, New York, and Connecticut.[1]

Nelson and Hedgcock examined a third-generation coppice growth at Mt. Alto, Pennsylvania, in 1928, and obtained cultures from decay in 17 of the trees. Sixteen of these cultures were *Stereum gausapatum*, which indicates that the fungus may be of increasing importance in sprout-grown oaks. Decay in these trees is thought to have gained entrance from the old stumps, and in all of the other cases, with the exception of 4 fire-scarred trees, the entrance of decay seemed to be from old stumps or through dead companion sprouts.

The height of the decay in the trees at Mt. Alto was not recorded, but observations by the writer in 1933 indicate that it advances well above stump height. In 4 cases the decay was taken from butt logs left as culls by logging crews.

Additional field studies are being conducted that should bring out more definitely the relative importance of this and other decay-producing organisms. *Stereum spadiceum* Fr., which is given as a synonym of *S. gausapatum* by Burt, is the cause of decay in standing oaks in France.[2]—ROSS W. DAVIDSON, Division of Forest Pathology, Bureau of Plant Industry, Washington, D. C.

Stem-spot of Rhubarb.—A disease of *Rheum rhaponticum* has been known in California since 1914, but to the writer's knowledge, has never before been reported. The disease is now prevalent in many rhubarb fields and is most severe on the first cuttings. It is characterized by oval to elliptical reddish or reddish brown spots 1–15 × 1–3 mm. in size, or larger when they coalesce. A species of Phyllosticta (tentatively determined as *Phyllosticta straminella* Bres.) has been repeatedly isolated from the lesion on the petioles. It is probable that the stem-spot herein described may be identical with this disease, as reported by Stevens.[1] He states that *P. strami-*

[1] The writer is indebted to A. D. Chapman, George G. Hedgcock, R. M. Nelson, L. W. R. Jackson, and P. Spaulding for collecting many of the specimens and to Chapman, Jackson, and Nelson for culturing some of them.

[2] Les pourritures du bois de chêne sur pied. Bull. 13, Comm. d'études des ennemis des arbres, des bois abattus et des bois mis en oeuvre. Ann. de l'École Nat. des Eaux et Forèts et de la Stat. de Recherches et Exp. Forest., iv, 2, pp. 365–380, 400–401, 406–407. 1932.

[1] Stevens, F. L. Two Illinois rhubarb diseases. Illinois Agr. Expt. Sta. Bul. 213: 299–312. 1919.

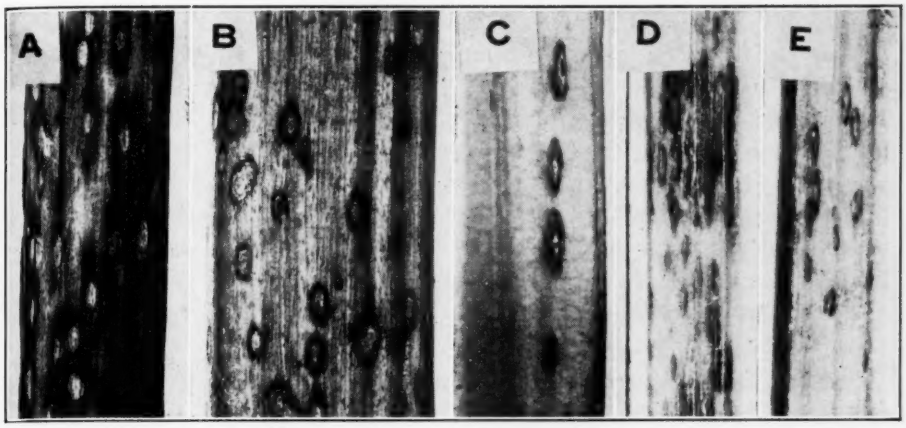

FIG. 1. A–B. Natural lesions on petioles of rhubarb. C. Inoculation through a wound using spores of *Phyllosticta straminella*. Photographed after 30 days. D–E. Stems inoculated with spores of *P. straminella* on cotton saturated with inoculum and wrapped about the petioles with paraffin paper. Photographed after 52 days.

nella, while mainly affecting the leaf blade, has been found also upon the petioles. He gives no illustration or description of these stem lesions. In California the stem-spots are of more economic importance, although the leaf-blade and veins are attacked and furnish abundant sources of inoculum for infecting the petioles. In 60 cultures made from the stem lesions, 35 gave *Phyllosticta straminella*. The other cultures were miscellaneous fungi, as Alternaria, Rhizopus, Cladosporium, and Penicillium. Artificial inoculations and controls on the petioles were made through wounds, by atomizing, and by placing cotton saturated with inoculum in contact with the uninjured petioles wrapped with paraffin paper. More or less typical lesions developed in 30 to 45 days. Through the courtesy of J. C. Gilman a herbarium specimen of *Phyllosticta straminella*, collected August 9, 1927, was received by the author November 22, 1932. This specimen was number 1624, United States Department of Agriculture, and Iowa State College Mycological and Plant Disease Survey of Iowa.[2] Eleven different dilution plates were made from this 5-year-old herbarium material and 524 colonies of *P. straminella* grew. Transfers from selected colonies were pathogenic on rhubarb. This paper is preliminary to a more detailed study.—CLAYTON O. SMITH, University of California, Citrus Experiment Station, Riverside, California.

Observations on Gymnosporangium germinale.—A dissemination distance for *Gymnosporangium germinale* (Schw.) Kern, the causal organism of quince rust, from *Juniperus communis* var. *depressa* Pursh to apples of certain varieties has been determined at Orono, Maine. The orchard under

[2] Gilman, J. C., and W. A. Archer. The fungi of Iowa parasitic on plants. Iowa State Col. Jour. Sci. **3**: 299–507. 1929 (See page 376.)

consideration is slightly west of north from a stand of heavily infected Juniperus. For 3 consecutive seasons fruit infected by the quince rust organism has been found throughout the Red Delicious part of the orchard. In a survey of the surrounding land, no other stand of Juniperus has been found. The approximate shortest distance between the infected Juniperus and infected apple trees is 3,900 feet. The extreme distance between the infected hosts is over 4,500 feet. The degree of fruit infection per tree varied from none to approximately 5 per cent by fruit count on the variety, Red Delicious, in 1933. Only an occasional tree with a poor fruit set escaped infection. The local weather data during the dissemination period are given below:

TABLE 1.—*The temperature extremes, precipitation, and direction of the wind for 8 consecutive days beginning May 24, 1933*

Date	Temperature degrees F.		Precipitation	Prevailing wind
	Maximum	Minimum		
			Inches	
May 24	74	48	0.14	S.
" 25	76	45	0.00	S.
" 26	86	48	0.00	S.E.
" 27	52	38	0.88	S.E.
" 28	51	38	0.00	N.
" 29	74	36	0.00	S.E.
" 30	55	46	0.00	S.E.
" 31	60	47	0.91	S.E.

The weather data coincide with those considered to be favorable for sporidia dissemination, germination, and infection of the host during at least part of the interval of maximum sporidia production and fruit susceptibility. The direction of the wind was relatively favorable for dissemination of sporidia from the infected Juniperus to the orchard under observation. During the past 3 years fruit of the following varieties of apples infected by *Gymnosporangium germinale* have been observed in Maine orchards: Baldwin, Bellflower, Cortland, Duchess, Early Red McIntosh, Golden Delicious, Gravenstein, Jonathan, McIntosh, Red Delicious, Starking, Tolman Sweet, Twenty Ounce, Wagener, Wealthy, and Winter Banana. In Maine the most susceptible commercial varieties apparently are Red Delicious, Golden Delicious, Cortland, and Wealthy. The region of infection is at or near the calyx end of the apple fruit. Lateral infections, such as are shown by Thomas and Mills,[1] have not been observed. Aeciospore production on the apple fruit is not common.—F. H. STEINMETZ and M. T. HILBORN, College of Agriculture, University of Maine, Orono, Maine.

[1] Thomas, H. E., and W. D. Mills. Three rust diseases of the apple. New York (Cornell) Agr. Expt. Sta. Mem. 123. Plate II. 1929.

REPORT OF THE ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The annual meeting of the Southern Division of the American Phytopathological Society was held in conjunction with that of the Association of Southern Agricultural Workers in the Peabody Hotel, Memphis, Tennessee, January 31 to February 2, 1934.

Three half-day sessions of the Society were scheduled but, on meeting the first day, it was found more convenient to combine the three scheduled programs into two and to terminate the meetings in so far as our Division was concerned on the afternoon of February 1.

At the business meeting, on the afternoon of January 31, the following officers were elected to serve the Society for the next year:

Chairman ..... D. C. NEAL, U. S. Cotton Breeding Station, Greenville, Texas.

Secretary-Treasurer ..... E. C. TIMS, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Councilor ..... V. H. YOUNG, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

This was the first meeting of the Southern Division to be held in conjunction with the meetings of the Association of Southern Agricultural Workers since such a practice was abandoned several years ago in favor of meetings to be held during the summer months. The summer meetings, however, were never very successful because of the difficulty experienced in securing an adequate attendance, most of the workers finding it difficult to leave their research projects during the summer months at the time when they were most active and required the closest supervision.

The attendance at this meeting was small, as was naturally to be expected after such an interval, and also because of the world-wide depression and its effect on the budgets for traveling purposes, which have been materially reduced if not entirely eliminated in all Southern institutions. In spite of the small number present, a total of 21 excellent papers were presented in a manner characterized by a delightful informality that contributed greatly to sustaining interest throughout the sessions. Ample opportunity was afforded for discussion, which was indulged in freely by everybody. Abstracts of the papers follow the present report.

L. E. MILES, *Secretary*

ABSTRACTS

Studies on Potato-Scab Control.—J. J. TAUBENHAUS. In previous experiments by the writer no appreciable control of potato scab (*Actinomyces scabies*) was obtained when sulphur was used at the rates of 1,000 to 5,000 pounds per acre and applied broadcast to soils rich in calcium and of an alkaline reaction. During 1933, a series of potato scab experiments with sulphur was carried out in different sections of East and East Central Texas and under a variety of soil conditions ranging from neutral to alkaline in nature. The sulphur in these experiments was applied in the furrows some two or three weeks before planting at rates varying from 500 pounds in the neutral soils to 1,500 pounds per acre in the calcareous soils. The potatoes were then cut and planted in the usual way and the crop was given ordinary field culture.

Definite control of potato scab was obtained in all the tests and under all the soil types where the sulphur was applied in the furrow. This control of potato scab in these experiments was independent of soil reaction, particularly in the calcareous soils where no change of pH was obtained when the sulphur was placed in the furrows. In this connection, it should be noted that slight reduction in yield of tubers was obtained in the sulphur-treated plats, although this was to a certain extent compensated by smoother and better quality No. 1 tubers where the sulphur was used.

Studies on Nailhead Spot of Tomatoes.—GEORGE F. WEBER. Nailhead spot is a common disease of tomatoes found in the Southeastern and Gulf States, causing considerable damage annually. Confusion manifested in the literature concerning the causal organism and certain other closely related organisms on tomatoes has prompted this study and the following conclusions: The fungus causing nailhead spot, known in literature as *Macrosporium tomato* Cke., has been shown through cultural studies to be a species of Alternaria, and the new combination, *Alternaria tomato* (Cke.) is proposed, It is morphologically and physiologically distinct from the early blight parasite. *Alternaria solani* (E. & M.) J. & G., with which it is often confused. Both parasites are found on individual tomato plants, and causes distinguishable, but similar, diseases on stems, foliage, and fruit. These parasites show notable differences and variability in pure culture. Spores collected in nature and those developed in pure culture are consistently comparable for each parasite, but distinct from each other. None of a number of other species of Alternaria isolated from tomatoes have proved to be parasitic through artificial inoculation.

Studies on the Control of the Seedling Blight of Rice in Arkansas.—E. M. CRALLEY. The seedling blights of rice are widely distributed throughout the rice-growing areas in Arkansas. Most of the blighting is due to *Helminthosporium oryzae* von Breda de Haan. In 1933 Fusarium and a nonfruiting, sclerotium-bearing fungus caused considerable blighting. Thus far the seedling blights have been controlled most effectively by (1) the use of good seed, and (2) by delaying the planting date until the soil temperature is favorable for the vigorous growth of the young rice seedlings. According to preliminary greenhouse and field experiments the most serious blighting takes place when the soil temperature is between 16° to 23° C. The seedling diseases were not satisfactorily controlled by treating rice seed with formaldehyde, Ceresan 1, Ceresan 2, or copper-lime dusts. No seedling blighting was observed in 1933 in experimental plots where the rice was sown early in the season directly in the water.

Treatment of Sweet-Potato Plants for the Control of Black Rot.—L. E. MILES. Spread of black rot on sweet-potato plants during transit is a cause of great loss to the grower of sweet-potato plants for the market, regardless of however careful and con-

scientious he may be in applying the control measures already in practice against the disease in the seed bed, field, and storage in that a single infected plant may often spread the infection throughout an entire shipment while the plants are in transit from grower to purchaser. Terminal inspections now in vogue in many States cause the condemnation and loss of such shipments; or, if they are not intercepted, the loss is suffered by the purchaser.

This loss, as shown by tests conducted at this station in 1932 and 1933, may be prevented by the grower by dipping the plants, prior to packing for shipment, into a 20–20–50 Bordeaux-mixture solution, the stems and roots alone being immersed, or by immersing the entire plants in a 25 per cent copper-lime dust. Infection of the plants by spores of the disease organism present on their surfaces is prevented. Such treatment, moreover, does not injure the plants materially, even when the treated plants are in shipment in a closed container as long as 5 days, as is shown by stand and yield records taken on treated and nontreated plants. Such treatment is recommended to the grower of plants for either sale purposes or for home use in order to prevent dissemination of the disease organism to clean plants through any chance infection that may be present in the seed bed. There is evidence that this treatment will delay infection from infested soil to an extent that may make its use practicable when clean plants are put out on land known or suspected to harbor the disease, though this phase of the work has not yet been sufficiently worked out.

Preliminary Report on an Anthracnose of Lima Bean.—T. D. PERSONS. On August 20, 1932, specimens of diseased Lima beans were received from Lyman, Mississippi. The diseased areas were reddish brown and of irregular outline. Their size varied from very small reddish specks to areas as wide as the width of the pod, and in some cases these areas had coalesced to such an extent that almost the entire pod was affected. There was an abundance of pink spore masses in the diseased areas, and examination with a hand lens revealed the presence of dark setae around the edges of the spore masses.

The fungus causing this disease has, apparently, not been definitely determined; it would seem, however, to be a Colletotrichum. In 1883, M. C. Cooke collected a fungus on Lima beans in South Carolina and described it as *Vermicularia polytricha*. His description in Grevillea, vol. 12, p. 24, though very meagre, fits this fungus very well, especially as to spore size and shape. His spore measurements are given as $30 \times 4\ \mu$. In 1911, Heald and Wolf described a fungus causing a stem canker of Kentucky Wonder beans in Texas as *Colletotrichum caulicolum*. Their description in Mycologia, vol. 3, p. 10, also fits this fungus very well. Their spore measurements are given as 18–30×3.5–$4\ \mu$. The size of the spores of the fungus causing the disease in Mississippi, according to measurements recently made, varies from 21.6 to $28.8\ \mu$ in length and the width in all cases was approximately $3.6\ \mu$. The spores in nearly all cases were more or less sickle-shape. In the Plant Disease Reporter, vol. XV, p. 113, W. D. Moore reports a pod blight of Lima beans in North Carolina and Virginia. His description of the symptoms on the pods agrees very closely with those of this disease. This report also calls attention to the resemblance between the causal fungus and the descriptions of *Vermicularia polytricha* and *Colletotrichum caulicolum*.

The writer plans to continue his studies of this disease in an effort to definitely identify the causal organism and to formulate control measures.

A Report on the Use of Creosote Oil to Control San Jose Scale and Peach Leaf Curl.—W. W. STANLEY, S. MARCOVITCH, and J. O. ANDES. Tests during the last two years have shown that creosote oil is toxic to San Jose scale and to peach-leaf curl. The

control obtained was in direct proportion to the amount of creosote oil in the spray. The strongest spray used was 8 per cent creosote oil, which gave 91.09 per cent control of San Jose scale and 96.54 per cent control of peach-leaf curl. Combinations of small amounts of creosote oil and oil emulsion were much more toxic than when the materials were used separately or at higher concentrations. The combination of 1 per cent creosote oil and 3 per cent oil emulsion gave 99.08 per cent control of San Jose scale and 97.96 per cent control of peach-leaf curl. Creosote oil and coal-tar distillate (Arbo) appeared to be equally toxic.

The creosote oil used in the tests is a product of destructive distillation of hardwoods and was obtained from the Tennessee Eastman Corporation, Kingsport, Tenn.

Recent Investigations of Cotton Root Rot in Texas.—D. C. NEAL. It has been determined that the fungus causing root rot overwinters in the soil chiefly in the sclerotial or strand-sclerotial stage, and as dormant mycelium in plant roots infected from the previous year's crop. Numerous tests have shown that the sclerotia and the strands are capable of persisting in the soil in a viable condition in the absence of live host material for long intervals, doubtless for several years, and that these stages of the pathogen occur in some areas at considerable depth in the soil.

Careful records of infection show that root-rot spots, though usually persisting in alfalfa or cotton fields for several years and enlarging each year by a new belt of growth, may sometimes disappear for a season or longer and then reappear as small centers of infection that begin to expand with renewed vigor. Recent studies of the growth of the fungus on culture media have shown that the above spot behavior may be associated with a more or less normal growth cycle of the fungus itself. For example, fresh isolations of the fungus usually make rapid growth and produce strands and sclerotia in abundance, whereas cultures maintained for several months frequently become attenuated or dissociated and lose their capacity to produce strands or scleortia and yield only fine white mycelium. Since it is definitely established, even in the case of isolations obtained from widely separated localities, that the fungus may become attenuated *in vitro*, it is thought that at some period of its growth it may assume a similar morphology in nature, a stage that undoubtedly would be unfavorable for prolonged viability in the soil, thereby resulting in a breaking up or disappearance of the infection.

Additional experiments conducted during the seasons of 1932 and 1933 with ammonia-nitrogen carriers have further confirmed the toxic effect of ammonia on the fungus. Also, in studies of the toxic effect of ammonia water on sclerotia, strands, and mycelium of the fungus in cotton-root sections, it was found that the sclerotia and the strands are killed after short exposure to 1 and 2 per cent solutions, but that a longer time and a higher concentration is required to kill the fungus completely in infected root sections. In field experiments groups of root-rot sclerotia buried in Wilson clay soil at a depth of 12 inches were killed with 3 per cent ammonia water after an exposure of 5 days, and in other groups similarly treated, but buried at a depth of 18 inches, the number remaining viable was perceptibly reduced.

Further observations on the effect of field applications of ammonium sulphate, ammonium hydroxide, anhydrous ammonia, and other ammonium compounds continue to show that ammonia is effective in reducing the disease in certain sections of the infested districts of Texas. In heavily infested plots treated in 1932 and 1933 wth ammonia and ammonium compounds, significant reduction of the disease was obtained, and the residual effect of the 1932 application was still marked during 1933. The most favorable results, however, both from the standpoint of increased yields of seed cotton and in a reduction of root rot have been obtained where ammoniacal materials were used in Wilson clay or related types of soil.

Reversible Vegetative Dissociation of Strains of Phymatotrichum omnivorum.—WALTER N. EZEKIEL and J. J. TAUBENHAUS. Strains of *Phymatotrichum omnivorum*, when transferred by portions of the hyphal mats in agar slants, have retained their original cultural characteristics for more than 5 years. It has been possible, however, regularly to select substrains of dissimilar characteristics, by growing the fungus in flask cultures and making repeated transfers, in one series using as the inoculum sclerotia from the first flasks to produce these bodies and in a parallel series using as inoculum portions of the hyphal mat from the last flask to produce sclerotia. Strains retained their characteristics when transferred from tube to tube; but further selection could still be made from them of new substrains that reverted to the original characteristics. These reversible dissociations from the strains may be considered as possible because of nuclear heterogeneity of the original mycelium, arising from previous anastomoses between various strains, or from occasional mutations.

Insects as Possible Distributing Agents of Cotton Root Rot Caused by Phymatotrichum omnivorum.—J. J. TAUBENHAUS and L. D. CHRISTENSON. A number of beetles, *Blapstimus fuscus*, and a species of Harpalus, which normally feed on cotton plants, were placed in screened cages in the laboratory and made to feed on cotton roots freshly infected by the root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar. These insects fed copiously on the infected cotton roots and on the Phymatotrichum strands. Likewise, a number of cotton-leaf-eating larvae were fed on cotton leaves that had been painted over with a heavy suspension of the spores of *P. omnivorum*. The fecal pellets or the entire insects were then surface sterilized in a bichloride solution and cultured on nutrient agar in Petri dishes. At no time was *P. omnivorum* recovered either from the fecal pellets or from the insects thus treated. From this it appears that cotton-feeding insects do not spread Phymatotrichum root rot of cotton.

Further Studies on the Toxic Principles that Determine Immunity of Monocotyledonous Plants from Phymatotrichum Root Rot. WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE. It has been shown that juices from the roots of monocotyledonous plants contain materials that inhibit growth of the root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar. Continued studies, directed toward isolation and identification of these toxic elements, have been carried out with fractions of juices, concentrated under reduced pressure and added to nutrient solutions. Ether extracts from the juices of 4 monocotyledonous plants (onions, corn, canna, and Hemerocallis) were generally toxic to the fungus. The aqueous residues invariably were toxic. Ether fractions from 4 dicotyledonous plants (carrots, turnips, potatoes, and sweet potatoes) were not toxic, except for potato juice at higher concentrations in one experiment, while the aqueous residues were toxic. These results suggest that the materials that make monocotyledonous plants immune from Phymatotrichum root rot are those present in the ether-soluble fractions of juices from the roots of these plants. The toxic elements in the autoclaved, concentrated, aqueous fractions are apparently present in comparable amounts in fractions from both susceptible and immune plants and presumably are not involved in the immunity of monocotyledonous plants.

Insects as Possible Distributing Agents of Cotton Wilt Caused by Fusarium vasinfectum.—J. J. TAUBENHAUS and L. D. CHRISTENSON. A number of cotton-feeding insects were brought into the laboratory and placed in screened cages containing roots, stems, or leaves from cotton plants definitely known to be affected by Fusarium wilt (*Fusarium vasinfectum* Atkinson). The fecal pellets from the insects were then surface-sterilized and cultured on nutrient agar in Petri dishes. Some entire insects, when gorged with

the infected cotton material, were cultured also in the same manner as the fecal pellets. Good growth of *Fusarium vasinfectum* was recovered from the entire insects or the fecal pellets of the following: white grubs (*Phyllophaga crassissima*), grasshoppers, stem borers (*Ataxia crypta*), boll weevil larvae (*Anthonomus grandis*), and boll worms (*Heliothis obsoleta*). No Fusarium growth was obtained from wire worms.

In other experiments cotton insects were fed on infected cotton material and, after 24 hours' feeding, were killed in a bichloride solution. The alimentary canal, aseptically dissected out, was then cut up in small portions and cultured. *F. vasinfectum* was recovered from all parts of the gorged alimentary canals of the larvae of *Schistocera americana* and *Alabama argillacea*. In similar experiments insects were fed on infected cotton material, then starved for several days and all the fecal material was defecated. These insects were then killed in bichloride solution, the alimentary canal aseptically dissected out, cut up in small portions, and cultured. No Fusarium growth was obtained from the sections of the alimentary canal void of infected cotton-plant food. This indicates that the fungus, *F. vasinfectum*, the cause of cotton wilt, is able to pass through, in a viable condition, the alimentary canal of certain cotton feeding insects. The fungus thus recovered was capable of infecting normal cotton plants. These experiments show that many cotton-feeding insects may act as disseminating agents of cotton wilt.

Studies on the Fusarium Wilt of Cotton.—V. H. YOUNG and J. O. WARE. Cotton wilt and "rust" or potash hunger are commonly associated on much of the sandy alluvial soil of Arkansas. Often root knot is a factor. Since cotton wilt becomes unusually severe when either root knot or rust is present, their control is prerequisite to cotton-wilt control. No better method of control for root knot has been found than the use of resistant or immune crops and weed control in rotation with cotton. Work at the Cotton Branch Experiment Station in Lee County in Eastern Arkansas and elsewhere in Central and Eastern Arkansas had demonstrated the efficiency of liberal applications of potash either in mixed fertilizer or alone in the form of muriate of potash or kainit for the control of cotton wilt and rust. Stable manure at the rate of 10 tons per acre gave excellent control of rust, but data concerning its effect on the incidence of cotton wilt were not clear cut. Cotton seed meal at the rate of 600 pounds per acre was entirely ineffective so far as either wilt or rust was concerned. Nitrates or phosphates either alone or used together not only gave no control of either potash hunger (rust) or wilt but actually appeared to unbalance conditions so that more rust and wilt resulted than was present on the unfertilized checks.

In 1933 cotton wilt was more severe than for several years. Unfertilized checks of susceptible varieties of cotton, such as Trice 304 and Missdel 2, showed about 36 per cent of plants affected with wilt. Use of 600 pounds per acre of a 6–8–12 (NPK) fertilizer gave complete control of potash hunger and reduced wilt to about 8 per cent when Trice 304 cotton was used, to about 16 per cent when Missdel 2 was used, and to about 2 per cent when Arkansas Rowden 2080, a wilt-resistant strain, was employed. Kainit or muriate of potash in amounts sufficient to supply potash in amounts mentioned above gave similar control of potash hunger and cotton wilt but gave lower yields than did a complete fertilizer. Results of 5 years' work on the control of wilt and rust indicate that commercial-cotton wilt and rust control on the sandy alluvial soil of Arkansas is best secured by the combination of a suitable wilt-resistant variety of cotton with the judicious use of a suitable fertilizer containing enough potash to supply the potash needs of the soil.

Seed-Treatment Studies with Fungicidal Dusts at the Arkansas Experiment Station.—V. H. YOUNG. Seed-treatment studies with oats and corn were carried out at the

Main Experiment Station in northwest Arkansas and seed treatments with cotton at the Main Station and at the Cotton Branch Station in eastern Arkansas.

Organic-mercury and formaldehyde dusts gave almost perfect control of oat smut (*Ustilago avenae* and *U. levis*) with corresponding yield increase. There also was some indication of other benefits than smut control. Slight stand increases resulted from treatment of apparently healthy seed of Neal's Paymaster Corn with various mercury-containing dusts when seed was planted early in the season; but later plantings were not benefitted and in no cases were yield increases closely correlated with increases in stand. It was concluded that corn-seed treatment, under the conditions of the experiment, were of little or no value. The oat- and corn-seed-treatment studies were the joint work of C. K. McClelland and the writer.

Earlier cotton-seed treatments made by the sulphuric acid-delinting method, combined in some cases with the use of mercuric chloride, hastened germination and gave excellent control of seed-borne diseases, but growers have been unwilling to adopt them. Later experiments with Germisan and Uspulun in solution gave good control of angular leaf spot of cotton, increased the stands, and appeared to benefit yields often as much as 20 per cent. Liquid treatments for cotton have never appeared acceptable to Arkansas growers and the work was discontinued.

During the past 4 years, organic-mercury dusts, formaldehyde dust, red oxide of copper, and monohydrated copper sulphate have been used, the two latter dusts only in 1933. Dusts containing ethyl mercury chloride and ethyl mercury phosphate have given consistent stand increases, especially with early planted cotton, often amounting to as much as 50 to 100 per cent, and have given considerable control of angular leaf spot of cotton. Other dusts employed were either detrimental to germination of cotton or had no discernible effect. In spite of stand increases from cotton-seed treatments, very few actual yield increases have been recorded. It is believed that failures to secure definite yield increases in correlation with stand increases is due to the unusual ability of the cotton plant to adjust itself to available space unless excessively long skips exist.

Observations on the Control of Black Rot of Grapes.—V. H. YOUNG. In the grape-growing section of northwest Arkansas, black rot, caused by *Guignardia bidwellii*, is the most serious disease of Concord grapes. Although a schedule of sprays consisting of 4–4–50 Bordeaux mixture just before blooming, just after blooming, and about 10 days or 2 weeks later has ordinarily controlled the disease, many growers formerly failed to secure control due in some cases to unusually unfavorable location of their vineyards and in others to poor spraying or improper timing. Belief that one or more additional preblossom sprays were necessary for black-rot control apparently arose from failure of these growers to control the disease and from the recommendations of certain eastern stations where an additional preblossom spray is often recommended. Spraying experiments, extending over the period 1929–1933 using from 1 to 3 preblossom sprays and heavier dosages of copper and lime than those found in a 4–4–50 Bordeaux mixture, gave no better control than was secured with the standard schedule recommended for Arkansas growers. Likewise, during the 5 years of the experiment a late, nonstaining spray consisting of 1 pound of neutral acetate of copper to 50 gallons of water did not improve control, since, in every case, black-rot spread was checked by the dry weather of midsummer, making late spraying for the disease unnecessary.

In 1930 severe freezing in midwinter resulted in much killing back of grape vines and a subsequent unusual development of suckers that arose at or often below the soil line. On May 23, 1930, large numbers of black-rot cankers were found at the base of these suckers. They were located in an area extending from a few inches above the soil

line to just below it and were covered with pycnidia containing mature spores. At this time black-rot lesions on the upper parts of the vines were just appearing and showed no fruiting bodies. It appears that infections on suckers must have resulted from spores washed onto the young sprouts just as they pushed from the soil. Since large numbers of black-rot mummies from the previous season were present on the ground, it is suggested that these unusually early infections probably arose from conidia or ascospores borne on mummies of the previous year. Rapid growth of a corky layer on the suckers soon isolated the diseased tissues and by midseason they had almost disappeared.

Control of Fire Blight.—H. R. ROSEN. Investigations on the possible prevention of fire blight by the application of sprays applied at times when bacterial dissemination occurs have been continued. As in 1932, excellent control was obtained in 1933 in a Jonathan orchard by the application of 1–3–50 Bordeaux mixture applied as follows: First, as a cluster-bud spray; secondly, when about 20 per cent of the blossoms were fully opened; thirdly, when about three fourths of the blossoms were fully open; fourthly, as a calyx spray; and fifthly, as a first-cover spray.

A brief summary of the results shows that on the 56 check trees that received lime-sulphur spray applied as a cluster-bud, calyx, and first cover spray, the average amount of blighted clusters per tree was 30 per cent, varying from a trace to destruction of almost all blossoms. On the 87 trees sprayed with Bordeaux mixture the amount of blossom blight was restricted to an insignificant fraction of 1 per cent.

Twig blight was negligible on the Bordeaux-sprayed trees, while on the check trees it was largely confined to sprouts that arose from blighted clusters.

Russeting of the fruit on the Bordeaux-sprayed trees was more abundant in 1933 than in 1932, although the amount of injury was not sufficient to reduce seriously the marketable value of the fruit.

High Points in Apple Spraying in Tennessee.—J. O. ANDES. Blossom blight was partially controlled by spraying with 1–3–50 Bordeaux mixture when the blossoms were two thirds open. Canker treatment as a supplementary measure, using a 53 per cent zinc chloride solution, is recommended. On Golden Delicious, flotation sulphur, 8 pounds to 50 gallons, was found an effective substitute for liquid lime-sulphur and Bordeaux mixture in scab control. Bordeaux mixture applied to fully grown fruit of this variety did not cause russeting. Up to 9 sprays was required to give adequate control of all diseases and insects on all varieties under all conditions. Some of the sprays can be omitted under certain conditions without a decrease in the effectiveness of control.

Control Measures for Rosette of Blackberries and Dewberries in Louisiana.—A. G. PLAKIDAS. Studies on rosette in Louisiana have shown that: (1) the cause of the disease is a species of Cercosporella; (2) infection takes place in the spring on the primocanes, the infected canes remaining normal in appearance until the following spring when, on resuming growth, they develop the rosette symptoms; (3) the period of infection is limited from about the middle of March to the first week in June; (4) the disease can be effectively controlled by spraying the primocanes with Bordeaux during the period of infection. From these findings, a system of control has been devised that, while still in the experimental stage, has proved very effective. This system is a combination of pruning and spraying. All the primocanes are pruned to the ground about the middle of May. Then, the new canes that develop from the pruning date to about the middle of June are sprayed with Bordeaux. Usually, two sprayings seem to suffice. Because of the long growing season in Louisiana (the plants continue to grow as late as Novem-

ber) the removal of the early primocanes apparently does not weaken the vigor of the plant.

Stilbum on Fig in Louisiana.—EUGENE C. TIMS. Cross inoculations made on other hosts with *Stilbum cinnabarinum* (Mont.) from fig indicate that the cultures used are capable of attacking pear (Pineapple variety) and to a limited extent rose (Killarney variety), but are not parasitic on plum, peach, or pecan. The Celeste fig is quite susceptible, but some of the larger, coarser varieties, such as the Brunswick, are more resistant. Infection of the fig may take place through wounds or leaf scars, as has been demonstrated by various types of inoculations. Pruning out infected branches and painting the cut ends with outside paint has not been effective in eliminating the fungus from one small group of fig trees. Other control measures are being tried.

New or Unusual Diseases Reported or Observed in Mississippi in Recent Years.—T. D. PERSONS. The following is a list of new or unusual diseases reported or observed in Mississippi in recent years by various workers. In some cases the diseases are new for the hosts, while in others they are known to occur in other parts of the country but have not previously been reported from this State.

Root knot, caused by the nematode, *Heterodera marioni*, has been collected on *Abelia* sp. and on *Ginkgo biloba*. This constitutes the first report of the occurrence of this trouble on either of these hosts. The occurrence on Ginkgo is especially interesting because of the unique botanical position of this plant. In so far as the writer has been able to determine, this is the first report of root knot on any of the gymnosperms in the United States. Southern blight (*Sclerotium rolfsii*) is reported for the first time on China aster. Bacterial spot (*Bacterium holci*) on corn. This is apparently the first report of this trouble outside of Iowa, whence the disease was described originally. Verticillium wilt (*Verticillium dahliae*) on cotton is now known to occur in 7 counties in the State. Crown gall (*Pseudomonas tumefaciens*) on *Juniperus sabina*. First report of the disease on this host. The only other conifer known to be attacked by this disease is *Cupressus arizonica*, reported from Arizona by Brown and Evans. Orange rust (*Gymnoconia interstitialis*) is reported for the first time on loganberry. *Cercospora cruenta* is reported for the first time on mung bean. The Ascochyta blight, caused by *Ascochyta pinodella*, is reported for the first time on English peas in Mississippi. A leaf spot (*Sphaeropsis salviae*) on *Salvia* sp. reported for the first time in the United States. A wilt, caused by *Fusarium oxysporum nicotianae*, on tobacco for the first time from the State. Leaf spot (*Glocosporium aleuriticum*) on tung oil, and bacterial blight (*Bacterium juglandis*) on walnut also constitute new records for the State.

Sorosphaera veronicae (Schr.) on Corn or Wall Speedwell, Veronica arvensis.—LEROY DONALD. On May 30, 1930, specimens of *Veronica arvensis* were encountered that had conspicuous galls on their leaves and stems at or near the ground, the most common form being that of swollen, stunted stems from which sprang a few small, deformed leaves. The oldest swellings had turned brown and exhibited a snuff-like appearance. Since the galls or tumors suggested injury by nematodes, microscopic examinations were made that revealed the presence of numerous sphaerical and hollow spore balls within the enlarged parenchyma cells of the host. Occasionally, oblong or ellipsoidal compound structures were observed that probably resulted from a fusion of several of the spore balls. The individual spores were wedge-shape and varied from 15–20 μ in size.

The fungus was identified as *Sorosphaera veronicae* Schröter. As founded by Schröter in 1886 the genus Sorosphaera included only one species, namely, this one. The

genus was listed by him as one of 4 genera under the Phytomyxinae. He recognized 3 host plants in Germany on which *Sorosphaera veronicae* was parasitic, namely *Veronica hederifolia*, *V. triphylla* and *V. chamaedrys*.

Later, in 1895, Rostrup found this species on *Veronica hederifolia* in Denmark. Trotter, in 1904, reported it in *V. arvensis* from Italy. Cooke and Schwartz, while conducting an investigation of the root fungi in ponds and marshes around London, found and described from roots of a number of different grasses growing there a second species of the genus Sorosphaera, which was named *Sorosphaera radicalis*.

As will be noted, all the previous work on the genus Sorosphaera, as far as can be ascertained, has been conducted in countries other than the United States. The presence of either of the 2 species comprising the genus has never been reported from this country. *Sorosphaera veronicae* may be listed as a new fungus for the United States.

Verticillium Wilt of Cotton in Greece.—L. E. MILES. Verticillium wilt of cotton, first recorded as occurring on cotton under field conditions by Sherbakoff in Tennessee in 1928, and now known to occur on cotton in Tennessee, Arkansas, Mississippi, and California, has recently made its appearance in Greece.

In recent correspondence with the writer, Dr. J. A. Sarejanni, Plant Pathologist at the Institut Phytopathologique Benaki, Kiphissia-Athenes, Greece, advises that in September, 1932, he isolated the disease organism in 19 out of 20 cultures from Copais, Greece, from cotton grown from seed imported in the spring of 1932 from North Carolina. In later correspondence in January, 1934, he advises further that he has isolated the organism from cotton from the north in Macedonia as well as from the south in continental Greece, and always from cotton from seed imported from the United States in 1932 or 1933. *Fusarium vasinfectum*, the organism causing the very similar wilt of cotton in the United States, has been isolated only once in Greece, and in that case also by Sarejanni in 1932 from cotton grown from seed of American origin.

Sarejanni is strongly of the opinion that the disease was introduced into Greece on seed imported from America and states that "I should feel secure in saying that the introduction of American seed has brought about heavy infections in the cotton sections (of Greece)." Up to the present time, however, neither he nor the American workers who have investigated the matter have been able to establish the presence of the organism either in or on the seed nor to demonstrate whether or not the trouble is seed-borne. Moreover, the disease has not yet been reported on cotton from North Carolina nor the other Atlantic Coastal States whence the cotton seed imported into Greece presumably originated.

Cultures of the fungus isolated from cotton in Greece have been received and are identical in the growth characters of the colonies and the morphological details of the fungus with those secured from cotton in Mississippi and Tennessee.

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